CANMET

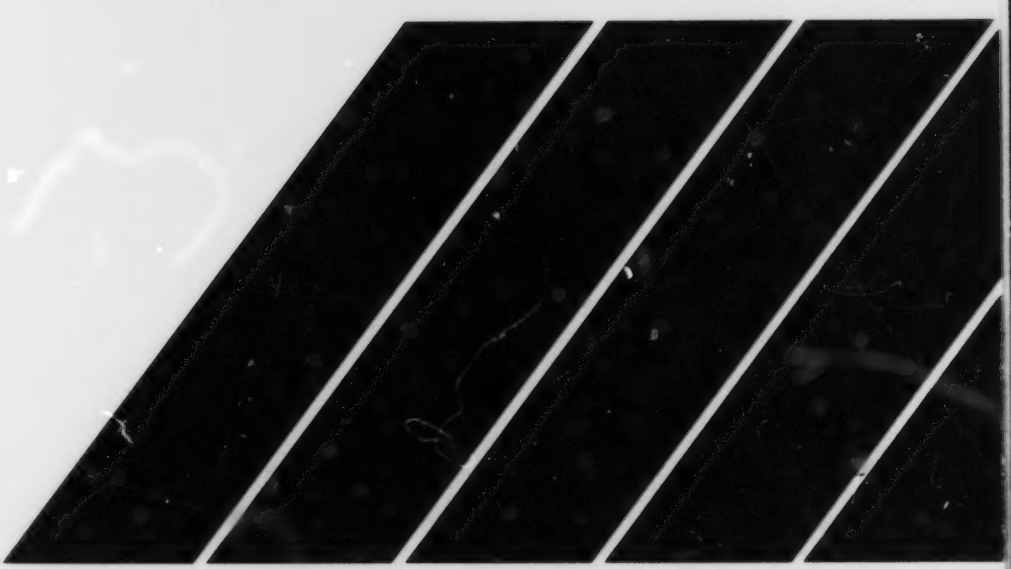
Canada Centre for Mineral and Energy Technology

Centre canadien de la technologie des minéraux et de l'énergie

Efficiency and Alternative Energy Technology Branch

Direction de la technologie de l'efficacité énergétique et des énergies de remplacement

CONTINUOUS CELL RECYCLE FERMENTATION



Energy, Mines and Resources Canada

Énergie, Mines et Ressources Canada

Canada

THE ENERGY OF OUR RESOURCES · THE POWER OF OUR IDEAS

L'ENERGIE DE NOS RESSOURCES · NOTRE FORCE CREATRICE

CONTINUOUS CELL RECYCLE FERMENTATION

PREPARED FOR:

Efficiency and Alternative Energy Technology Branch
Energy, Mines and Resources Canada
Ottawa, Ont.
DSS File No. 23440-0-9569
October, 1991

PREPARED BY:

University of Saskatchewan
Department of Chemical Engineering
Saskatoon, Sask. S7N 0W0

SCIENTIFIC AUTHORITY:

Sheldon Duff
Efficiency and Alternative Energy Technology Branch
Energy, Mines and Resources Canada
580 Booth Street
Ottawa, Ont. K1A 0E4

54 pp

MICROMEDIA

MICROMEDIA

92-06732/

CITATION

Warren, R.K., G.A. Hill and D.G. MacDonald, Dept. of Chemical Engineering, University of Saskatchewan. *Continuous Cell Recycle Fermentation.* DSS Contract No. 23440-0-9569. Efficiency and Alternative Energy Technology Branch, Energy, Mines and Resources Canada, Ottawa, Ontario. 1991 (54 pp.)

Copies of this report may be obtained through the following:

Efficiency and Alternative Energy Technology Branch
Energy, Mines and Resources Canada
580 Booth Street, 9th Floor
Ottawa, Ont.
K1A 0E4

or

Document Delivery Service
Library and Documentation Services Division
CANMET
Energy, Mines and Resources Canada
562 Booth Street
Ottawa, Ont.
K1A 0G1

DISCLAIMER

This report is distributed for informational purposes only and does not necessarily reflect the views of the Government of Canada nor constitute an endorsement of any commercial product or person. Neither Canada nor its ministers, officers, employees or agents makes any warranty in respect to this report or assumes any liability arising out of this report.

NOTE

Funding for this project was provided by the Federal Panel on Energy Research and Development, Energy, Mines and Resources Canada.

© Minister of Supply & Services Canada 1992
Catalogue No. M91-7/180-1992E
ISBN. 0-662-19742-9

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support of Energy, Mines and Resources Canada. Special thanks are given to Dr. Sheldon Duff in this regard. Also, the technical assistance and dedication of the summer students, Mr. Wenbo Hu and Ms. Kavitha Advikolanu, helped to make this work successful.

ABSTRACT

A cell recycle fermentor using a cross-flow membrane filter has been operated for extended periods. Productivities as high as 70 g/L/h were obtained at a cell concentration of 120 g/L and a product concentration of 70 g/L. The experimental results were then fitted to previously derived biokinetic models (Warren et al., 1990) for a CSTF. A good fit for growth rate was found and the cell yield was shown to decrease with product concentration. The product yield, however, was found to remain nearly constant at all cell, substrate and product concentrations.

These biokinetics, along with a previous model for the membrane filter (Warren et al., 1991) were then used in a simulation to estimate the costs of producing ethanol in a large scale system. This simulation was optimized using a variant of the steepest descent method from which a fermentor inlet substrate concentration of 150 g/L and net cost of production of $CAN 253.5/1000 L of ethanol were projected.

From a sensitivity analysis, the yield parameters were found to have the greatest effect on ethanol net cost of the fermentor parameters, while of the operating costs, the profit was found to be most sensitive to the wheat raw material cost and to the dried grains by-product value.

CONTENTS

ACKNOWLEDGEMENTS (i)

ABSTRACT (ii)

CONTENTS (iii)

EXECUTIVE SUMMARY (iv)

RESUME (vii)

NOMENCLATURE (x)

1. INTRODUCTION 1

2. OBJECTIVES 4

3. LITERATURE SURVEY 5
   - 3.1 Industrial Continuous Ethanol Processes 5
   - 3.2 Continuous Stirred Tank Fermentor with Recycle Kinetics 7
   - 3.3 Simulation and Optimization of a Cell Recycle Fermentor 11

4. THEORY 16
   - 4.1 Cell Recycle Kinetics 16
   - 4.2 Cell Recycle Fermentor Simulation 18
   - 4.3 Economic Optimization of the Cell Recycle System 21

5. METHODS 24
   - 5.1 Experimental Equipment 24
   - 5.2 Organism and Media 27
   - 5.3 System Operation 28
   - 5.4 Filter Backflushing 29
   - 5.5 Analytical Methods 30
   - 5.6 Fermentor Model Parameter Estimation 31
   - 5.7 Simulation, Costing and Optimization 32

6. RESULTS & DISCUSSION 34
   - 6.1 Cell Recycle Fermentor Kinetics 34
   - 6.2 Cell Recycle Simulation & Costing 43
   - 6.3 Cell Recycle Economic Optimization 44

7. CONCLUSIONS & RECOMMENDATIONS 50

REFERENCES 52

APPENDIX A. PRELIMINARY INVESTIGATION INTO RECYCLE FERMENTOR SIMULATION - IMPROVED BIOREACTION KINETICS FOR THE SIMULATION OF ETHANOL FERMENTATION BY *SACCHAROMYCES CEREVISIAE*.

APPENDIX B. PRELIMINARY DESIGN & COSTING OF A CONTINUOUS ETHANOL PROCESS FOR WESTERN CANADA USING CELL RECYCLE

EXECUTIVE SUMMARY

The fermentation of biomass sugars to produce ethanol has already become a large-scale, commercial industry to provide both an alternative motor vehicle fuel (gasohol) and to reduce the rate of gaseous pollution emissions caused by burning gasoline. All existing processes in Canada, and most processes in other parts of the world, rely on batch fermentation. Continuous fermentation, however, holds the promise of improving process efficiency by reducing fermentation volumes to produce a given amount of ethanol and by reducing manpower requirements. Most large scale chemical industries use continuous operation for these reasons.

This report describes an intensive study of the recycle, continuous fermentation process to produce ethanol. Both a theoretical modelling study and an experimental steady state study are reported. The design and economic evaluation of a large scale, continuous ethanol process are also included.

The theoretical modelling involves the development of some new equations and new best-fit parameters describing both the basic biokinetics of growth of *Saccharomyces cerevisiae* at high cell/ethanol concentrations and the steady state performance of the recycle fermentor at various bleed ratio (withdrawal rate of cells from the fermentor) conditions. It has been found that the product yield of ethanol remains constant under all operating conditions at the value Y_{ps} = 0.453 g ethanol/g glucose. The cell yield, however, was found to decline linearly with ethanol concentration starting from a maximum value of 0.082 g DW/L at zero ethanol concentration and falling to about 0.055 at 80 g/L ethanol concentration. The specific growth rate of the yeast was also best fit to

a linear product inhibition model with a maximum growth rate of 0.4 h^{-1} and falling to zero growth at 95 g/L ethanol concentration. It is shown that recycle fermentation modelling requires use of an intrinsic (taking into account the volume of the cells in the fermentor) set of equations to accurately predict the performance of this fermentation process. Simulations using this approach (see Appendix A) show that recycle fermentation should be carried out with an external bleed (cells withdrawn after the cell separator) and that low bleed ratios can reduce the need for high cell recycle while maintaining a high level of ethanol productivity.

The experimental work demonstrates the feasibility of long term operation of the cell recycle, fermentation process. The cells were observed to maintain a high level of viability at or above 90% under all operating conditions. Successful, continuous ethanol production was achieved even at cell concentrations of 120 g/L with an ethanol productivity near 70 g/L/h (as compared to a straight CSTF value of only 5 g/L/h). The hollow-fibre membrane filters provided long term, completely sterile cell separations. Flux decreases due to slow cell build-up on the membranes were overcome by the development of an on-line, back-flushing strategy combined with the operation of several filtration units in parallel. Good control of the filtrate flow was achieved by incorporating a novel, gas separation vessel in the filtrate line to remove carbon dioxide which penetrated the filter membranes. All the experimental data was found to follow expected trends for yields and growth kinetics. Negligible cell toxicity was found and it is suspected this was due to the provision of adequate levels of nutrients and the

bleed of toxic secondary products from the system.

Finally, the industrial, economic design demonstrates that at current prices, the continuous cell-recycle fermentation process could produce ethanol from Canadian wheat at a profit generating rate of 6 million dollars per year, for a plant producing 100 million litres per year of 99.2% ethanol. The sugar concentration feed to a continuous fermentor is shown to be optimum at 150 g/L while the fermentor should only be operated with a cell concentration around 50 g DW/L. These conditions should be easy to maintain in a large-scale continuous process.

The success of this study clearly demonstrates the desirability of commencing large-scale investigations. The new, larger diameter hollow-fiber membranes should be combined with a 20 to 50 litre fermentor to demonstrate the feasibility of scale-up. Even such a small pilot will be generating gasohol at a rate of up to 100 litres of pure ethanol every day or 35,000 litres per year. Production at such high levels should also be attempted with real wheat hydrolysates and other commercial types of nutrients to achieve the most economically efficient fermentation conditions.

RÉSUMÉ

La fermentation des sucres biomassiques utilisée pour produire de l'éthanol est déjà devenue une industrie commerciale de grande envergure qui offre une solution de remplacement au carburant pour les véhicules automobiles (essence-alcool) et permet de réduire le taux des émissions de gaz polluants causées par la combustion de l'essence. Tous les procédés existant au Canada ainsi que la plupart des procédés utilisés à travers le monde sont basés sur la fermentation discontinue. Cependant, en utilisant la fermentation continue, on améliorerait l'efficacité du procédé car les volumes de fermentation produisant une quantité donnée d'éthanol et la main d'oeuvre nécessaire seraient moindres. Pour ces raisons, la plupart des entreprises chimiques font appel à la production en mode continu.

Dans le présent rapport, on décrit une étude intensive du procédé de fermentation continue avec recyclage visant à produire de l'éthanol. On présente une étude de modélisation théorique et une étude expérimentale à l'état constant. On a également inclus la description de la conception et l'évaluation économique d'un procédé de production de l'éthanol en continu, à grande échelle.

La modélisation théorique comporte la mise au point de nouvelles équations et de nouveaux paramètres pour un meilleur ajustement aux données, décrivant à la fois la biocinétique de base de la croissance de Saccharomyces cerevisiae en présence de concentrations élevées de cellules/éthanol ainsi que la performance à l'état constant du fermenteur à recyclage pour divers rapports de purge (vitesse de sortie des cellules du fermenteur). On a constaté que le rendement d'éthanol restait constant dans toutes les conditions de fonctionnement à la valeur Y_{ps} = 0,453 g d'éthanol/g glucose. Le rendement des cellules diminuait toutefois linéairement en fonction de la concentration d'éthanol. Il commençait à une valeur maximale de 0,082 g masse sèche/L pour une concentration nulle d'éthanol et tombait à environ 0,055 pour une concentration d'éthanol de 80 g/L. Le taux de croissance spécifique de la levure était le mieux représenté par un modèle d'inhibition linéaire avec un taux maximum de croissance de 0,4 h^{-1} tombant à zéro pour une

concentration d'éthanol de 95 g/L. On montre que la modélisation de la fermentation avec recyclage nécessite l'application d'une série d'équations intrinsèques (prenant en compte le volume des cellules dans le fermenteur) pour prévoir avec exactitude la performance de ce procédé de fermentation. Des simulations basées sur cette approche (voir l'Annexe A) montrent que la fermentation avec recyclage doit être effectuée avec une purge externe (cellules retirées après le séparateur de cellules) et que de faibles rapports de purge peuvent réduire le taux de recyclage des cellules nécessaire tout en maintenant un niveau élevé de productivité d'éthanol.

Les travaux expérimentaux montrent que le procédé de fermentation avec recyclage des cellules peut fonctionner longtemps. On a observé que les cellules maintenaient un niveau élevé de viabilité, égal ou supérieur à 90 %, dans toutes les conditions de fonctionnement. On a réussi à obtenir une production continue d'éthanol même lorsque la concentration des cellules étaient de 120 g/L, la productivité d'éthanol étant de près de 70 g/L/h (comparativement à une valeur CSTF de seulement 5 g/L/h). Les filtres à membrane à fibres creuses permettent de séparer les cellules complètement stériles pendant longtemps. Pour éviter les diminutions du flux dues à une lente accumulation des cellules sur les membranes, on a mis au point une stratégie de purge en retour en ligne combinée à l'utilisation de plusieurs unités de filtration en parallèle. On a réussi à bien réguler le débit du filtrat en incorporant un nouveau dispositif de séparation gazeuse dans le tuyau à filtrat pour éliminer le gaz carbonique qui pénétrait dans les membranes filtrantes. On a constaté que toutes les données expérimentales concordaient avec les tendances prévues en ce qui concerne les rendements et la cinétique de croissance. La toxicité à l'égard des cellules s'est révélée négligeable et l'on soupçonne que cela est dû au fait que l'on a fourni suffisamment d'éléments nutritifs et que l'on a purgé les produits secondaires toxiques du système.

Finalement, l'étude de conception industrielle économique montre que compte tenu des prix actuels, le procédé continu de fermentation avec recyclage des cellules pourrait permettre de produire de l'éthanol à partir du blé canadien avec des profits de 6 millions de dollars par année dans le cas d'une usine produisant 100 millions de litres par année d'éthanol à 99,2 %. La concentration optimale de sucre dans un fermenteur continu est de 150 g/L et il ne doit

fonctionner qu'avec une concentration de cellules d'environ 50 g masse sèche/L. Ces conditions doivent être faciles à maintenir dans le cas d'un procédé continu à grande échelle.

Le succès de cette étude montre clairement à quel point il est souhaitable d'entreprendre des recherches à grande échelle. Les nouvelles membranes à fibres creuses de plus gros diamètre devraient être combinées à des fermenteurs de 20 à 50 litres pour que l'on puisse montrer la faisabilité de la mise à l'échelle. Même dans le cas d'un petit projet pilote comme celui-ci, on produira de l'essence-alcool à raison de 100 litres d'éthanol pur chaque jour ou 35 000 litres par année. La production de telles quantités devrait aussi être expérimentée avec des hydrolysats réels de blé et d'autres types commerciaux d'éléments nutritifs afin que l'on puisse trouver les conditions de fermentation les plus économiquement efficaces.

NOMENCLATURE

Generally, lower case letters indicate actual quantities and capital letters indicate dimensionless quantities.

a	Product inhibition power for cell growth
A	Membrane area available to filtration (m^2)
b	Bleed stream flowrate (L/h)
B	Ratio of bleed flowrate stream to feed stream flowrate (b/f)
C_{S1}	Constant in the substrate and substrate prepartion cost function
C_{F1}	Constant in the fermentor cost function equation
C_{M1} - C_{M3}	Constants in the membrane filter cost function equation
C_{D0} - C_{D6}	Constants in the distillation, evaporating and drying cost function equation
C_{DIST}	Cost associated with distillation, evaporating and drying ($/h)
C_{FERM}	Cost associated with the fermentor ($/h)
C_{MEMB}	Cost associated with the membrane ($/h)
C_{SUBS}	Cost associated with the wheat and its hydrolysation ($/h)
d	Dilution Rate (f_F/V_r)
D	Dimensionless dilution rate ($f_F/(V_r*\mu_m)$)
e	Filtrate stream flowrate (L/h)
E	Ratio of filtrate stream flowrate to feed stream flowrate (e/f)
f_{CR}	Cross-flow flowrate in the filter (m^3/h)
f_F	Feed flowrate into the fermentor (L/h for laboratory work, m^3/h for design work)
j_{AV}	Average flux through the membrane ($m^3/m^2/h$)
k_1	Exponential inhibition constant for growth rate equation (L/g)

k_2 Exponential inhibition constant for specific ethanol production rate equation (L/g)

K_2 Ratio of product inhibition constant for growth rate to that for cell yield (p_{m1}/p_{m2})

K_3 Ratio of product inhibition constant for growth rate to that for product yield (p_{m1}/p_{m3})

K_{M1} Pressure dependence of the filter cake resistance

K_{M2} Shear dependence of the filter cake resistance

K_{M3} Cell concentration dependence of the filter cake resistance

K_{M4} Flowrate dependence of the pressure drop along the filter

K_{M5} Cell concentration constant for the pressure drop along the filter

K_s Monod constant for growth rate (g/L)

p Fermentor abiotic-phase product concentration (g/L)

P Dimensionless fermentor product concentration (p/p_{m1})

p_{m1} Maximum abiotic phase product concentration for cell growth (g/L)

p_{m2} Product inhibition constant for $Y_{X/S}$ (g/L)

p_{m3} Product inhibition constant for $Y_{X/P}$ (g/L)

p_{m4} Product inhibition constant for $Y_{P/S}$ (g/L)

r Cell recycle stream flowrate (L/h)

r_x Rate of Cell Formation (g/L/h)

r_d Rate of Cell Death (g/L/h)

r_s Rate of Substrate consumption (g/L/h)

r_p Rate of product formation (g/L/h)

R Ratio of cell recycle to feed stream flowrate (r/f)

s Fermentor abiotic-phase substrate concentration (g/L)

S Dimensionless fermentor substrate concentration (s/K_s)

t	Time (h)
T	Dimensionless time ($t*\mu_m$)
V_b	Fractional cell viability
V_F	Fermentor working volume (L for laboratory work, m^3 for plant design work)
x	Fermentor cell dry weight concentration (g/L)
x_v	Viable cell dry weight concentration (g/L)
X	Dimensionless fermentor cell concentration (x/ρ)
x_b	Bleed stream cell dry weight concentration (g/L)
$Y_{P/S}$	Product mass yield from substrate (g/g)
$Y_{X/P}$	Mass ratio of cell dry weight yield to product mass yield (g/g)
$Y_{X/S}$	Cell dry weight yield from substrate (g/g)

GREEK SYMBOLS

γ	Shear rate (1/s)
κ	Ratio of cell dry weight concentration in the bleed stream to that in the fermentor (x_b/x)
μ	Specific growth rate (/h)
ν	Specific ethanol production rate (g/g/h)
π	Dimensionless constant ($\rho/(Y_{X/P}*p_m)$)
ρ	Cell dry weight per unit volume wet biomass (g/L)
ϕ	Dimensionless constant ($\rho/(Y_{X/S}*K_s)$)

CYRILLIC SYMBOLS

P	Pressure (Pa)

SUBSCRIPTS

0	Value at zero product concentration
E	Filtrate
I	Inlet
m	Maximum possible value
M	Membrane
GUESS	Initial guess of variable

1. INTRODUCTION

Fermentation ethanol has been produced as a beverage for centuries, but in recent years it has also attracted considerable interest as a transportation fuel. This was initially due to the oil crisis, but has since been spurred on by the need to find cleaner renewable fuels. In 1989, an estimated 12 billion L/yr of fuel ethanol were produced in Brazil (Laluce, 1991) and 3.2 billion L/yr in the U.S.A. (Wyman and Hinman, 1991). However, its production has only been possible thanks to government subsidies and tax exemptions (Kane and Reilly, 1989). Although ethanol's prospects should become more attractive in the long-term, as gasoline reserves continue to decline, the key to allowing it to compete economically in the short term is most likely to be in improved processing technology. In some of the newer plants, one area in which this has occurred is the use of continuous fermentation. This has allowed significantly lower fermentor volumes compared to plants using batch technology, as well as smoother overall operation. The continuous fermentors presently in operation are mostly cascade systems (Allen, 1982) where a series of continuously stirred tank fermentors are used, each with progressively higher ethanol concentrations. Although these systems reduce the effects of product inhibition, they still have low fermentor cell concentrations, thereby limiting the dilution rate and hence the productivity which can be obtained. It follows that the next development from this is higher cell concentrations, which, because of higher productivities, would allow the fermentor volume and cost to be further reduced.

Two approaches, cell immobilization and cell recycle, have been

suggested to obtain higher cell concentrations. A good review of bench-top immobilization systems is given by Godia et al. (1987). Semi-commercial production of ethanol using cells entrapped in calcium alginate has been reported (Samejima et al., 1984), but no commercial immobilized cell ethanol plants exist in North America.

The alternative to immobilization is cell recycle, in which the cells are separated after fermentation and then returned to the fermentor. Three general methods have been suggested for separating the cells - sedimentation, centrifugation and filtration. The initial publications primarily considered sedimentation, (Cysewski and Wilke, 1977; Ghose and Tyagi, 1979; Boks and Eybergen, 1981; Kuriyama et al., 1985). By its nature, settling produces an imperfect separation, especially as the liquid velocity in the settler is increased. Cooling may also be required in the settler to help the cells sediment. Because of these drawbacks, this method has not been used on a large-scale. Current commercial cell recycle systems, such as the Biostill process (Cook et al. 1984), use centrifugation. However, because continuous centrifuges are very expensive, most of the recent experimental work on cell recycle has been with membrane filters (Charley et al,1983; Cheryan and Mehaia,1983,1984; Lafforgue et al., 1984; Lee and Chang, 1987). These devices allow sterile separation with very high recovery efficiencies. The system is also a lot simpler to operate than a centrifuge and has the potential to be considerably cheaper.

The primary aim of this research was to study cell recycle using a cross-flow microfilter as a possible step in improving the economics of producing ethanol from the excess grains presently found in the North

American Mid-West. The report includes a study of the kinetics of a continuous stirred tank fermentor (CSTF) with cell recycle, and the economic optimization of a future large-scale, cell recycle ethanol plant using a simulation based on the experimental kinetics.

2. OBJECTIVES

The primary aim of this research was to study cell recycle using a cross-flow microfilter as a method to improve the economics of producing ethanol from wheat. The project focuses on two areas; i) the construction and operation of a bench-scale, high cell density continuous stirred tank fermentor and hollow-fibre filter, and ii) the development of a computer model to predict optimum economic operating conditions for an industrial scale cell recycle fermentor based on the kinetics found in the lab-scale studies.

3. LITERATURE SURVEY

3.1 Industrial Continuous Ethanol Processes

Ethanol has been produced by batch processes for thousands of years, with the technology changing little until the 1930s, when innovations such as reusing the yeast from the previous batch were established (Guidoboni, 1984). After the second World War, with the growth of the petrochemical industry and production of low cost, synthetic ethanol, fermentation ethanol production became increasingly limited to the beverage industry.

With the 1973 oil crisis, a surge of interest was generated in the use of ethanol as a gasoline extender. A study by Katzen and Assoc. (1978) evaluated the technical and economic feasibility for a 190 million L/year ethanol production plant using corn as a feedstock. The process suggested was continuous except for batch fungal amylase and fermentation sections. The annual operating cost was estimated at $U.S. 44.5 million, with a total production cost of $U.S. 0.23 /L ethanol. A selling price of $U.S. 0.27 /L ethanol was required to give a 15 % DCF-IR over a 20 year plant life based on an estimated capital investment of $U.S. 63.6 million. Using wheat increased this selling price to $U.S. 0.35 /L. A sensitivity analysis showed that the only significant variable that affected operating costs was the corn price, with the selling price of dry grains having a smaller secondary effect.

While most of the newly commissioned plants used traditional batch fermentation, systems using continuous fermentation were also suggested and built. A summary of some of these processes is given in Table 1 and a review of many of these processes is given by Guidoboni (1984). Many of these designs were for complete plants and included thermal integration.

Table 1 Industrial scale continuous fermentation processes used to produce ethanol.

PROCESS AND MAIN REFERENCE(S)	NO. OF FERM-ENTORS	CELL RECYCLE DEVICE	TOTAL FERM. VOL. (M^3)	OVERALL RESIDEN-CE TIME (h)	TEMP. (^{o}c) AND pH	INLET SUGAR CONCN. (g/L)	OUTLET CELL CONCN. (g/L)	OUTLET ETHANOL CONCN. (g/L)	SPECIAL FEATURES
BELLINGHAM PLANT (ERICSON, 1947)	8	CENTRI-FUGE		20	30 4.5	18	10	10	USES SULPHITE WASTE LIQUOR.
TOKYO PILOT PLANT (KARAKI ET AL., 1972)	6	NONE	600	50	30	204		86	USES THAI MOLASSES.
MOSCOW PILOT PLANT (YAROVENKO, 1978)	6	FILTER		17	 4.2	157	10	65	VALUES GIVEN FOR STARCH. FOR NON-ASEPTIC FEED, FERMENTOR MUST BE STERILIZED EVERY 2-3 DAYS.
DANISH DISTILLERIES (ROSEN, 1978)	2	CENTRI-FUGE	340	21	35 4.7	158	10	66	USES BEET MOLASSES. YEAST ACID WASHED BEFORE RECYCLED. IF RES. TIME <16 h FERMN. INCOMPLETE.
HOESCHT-UHDE (FAUST AND KNECHT, 1979)	1	SETTLER	1	3	37 4.5 - 5.0	140	50-60	70	USES UNSTERILIZED MOLASSES AND THERMO-TOLERANT YEAST. AERATED 0.05 VVM. 2ND SETTLER SEPARATES YEAST BY-PRODUCT. SINCE USED ON LARGER SCALE.
PROCESS ENG. CO. (LOSER, 1980)	1	CENTRI-FUGE							USES CORN. SERIES OF BY-PRODUCTS SEPARATED BEFORE FERMENTATION. 30 % BACKSET. STILLAGE CONVERTED TO METHANE. 180 % ENERGY EFFICIENCY.
VOGELBUSCH (ALLEN, 1982; 1984)	6	CENTRI-FUGE		24			25	78	USES CORN. FIRST 3 FERMENTORS AERATED. YEAST ACID WASHED BEFORE RECYCLED. 60 % BACKSET. NOTE THAT RES. TIME INCLUDES SACCHARIFICATION
ATPAL (GROUT AND ENGLISH, 1981)	1	CENTRI-FUGE				300	> 20		USES UNSTERILIZED MOLASSES OR CANE JUICE. INTEGRATED PROCESS USING FLASH VESSEL.
BIOSTILL (COOK ET AL., 1984)	1	CENTRI-FUGE	90	6-7	32 4.5			45 - 60	USES PRE-SACCHARIFIED WHEAT. FIBRE SEPARATED IN BENT SIEVE BEFORE CENTRIFUGE. STILLAGE >28 % SOL. RATIO PROCESS WATER TO WHEAT 0.5:1.
ALCON (GUIDOBONI, 1984)	1	SETTLER		7			15 - 45		USES ANY CLEAN FERMENTABLE SUGAR FEEDSTOCK. STERILE AIR ADDED.
TATE AND LYLE (GUIDOBONI, 1984)	1	SETTLER							USES ANY CLARIFIED UNSTERILIZED FEEDSTOCK WITH HIGH ALCOHOL TOLERANT STRAIN.
TECHNIPETROL (GUIDOBONI, 1984)	4	CENTRI-FUGE		24				62	USES BLACKSTRAP MOLASSES. STERILIZED AIR ADDED. YEAST ACID-TREATED BEFORE RECYCLE.
INRA (LEU-LLIETTE ET AL., 1984)	1	CENTRI-FUGE	400	12	32 3.3	160	10	59	USES SUGAR BEET. AERATION RATE OF 0.3 V/V/h ALLOWED RESIDENCE TIME TO BE DECREASED.

Details on the economics of some of these processes are given in Table 2. For dry-milling, the Process Engineering Company process was also significantly different in that it involved the separation of a number of co-products before fermentation and the conversion of the stillage to methane. This lowered the yield, assuming the corn was 63 % starch, from 0.39 to 0.36 L ethanol/kg corn, but according to Loser (1980) this was more than offset by high quality by-products. The conversion of stillage to methane also produced energy and saved significant amounts of energy otherwise required for evaporation and drying.

Table 2. Energy costs and estimated operating cost per L of ethanol.

PROCESS AND MAIN REFERENCE	FEED-STOCK	PLANT CAPACITY (10^6 L/YR)	ENERGY USED IN PROCESS (MJ/L)	OPERATING COST ($US/L)
PROCESS ENG CO. (LOSER, 1980)	CORN	190	5.0	0.16 (HUNT 1981)
VOGELBUSCH (ALLEN 1984)	CORN	230	7.1	0.10 (ALLEN 1981)
ATPAL (GROUT AND ENGLISH, 1981)	SUGAR BEET		13.4	
BIOSTILL (COOK ET AL., 1984)	WHEAT	7	8.4	

3.2 Continuous Stirred Tank Fermentor with Recycle Kinetics

The first reference to the kinetics of cell recycle systems for ethanol production used a flocculating cell system with cooling (Cysewski and Wilke, 1977). Operating with concentrations up to 50 g/L, no losses in cell viabilities or specific productivities were found compared to a CSTF, so that similar kinetics could be used. However, above a dilution rate of 0.75 h^{-1}, the settler capacity was exceeded, causing the cell concentration to decrease.

Ghose and Tyagi (1979), also used a cell settling system and obtained cell concentrations around 32 g/L at dilution rates below 0.25 h^{-1}. Above this dilution rate, the liquid velocity in the settler exceeded the yeast settling velocity so that the cell concentration dropped off rapidly.

In comparing the kinetics of a settler cell recycle system with kinetics obtained from a straight CSTF, Limtong et al. (1984) found agreement up to cell concentrations of 92 g/L and dilution rates up to 0.5 h^{-1}. The differences above this dilution rate were credited to incomplete separation of the cells.

Using non-growing cells with total recycle and a hollow-fibre microfilter, Nishizawa et al. (1983) found a constant ethanol yield of 0.43 g/g up to a dilution rate of 0.35 h^{-1}, above which the yield decreased. Cell viability was found to be always over 90 %, but the non-growing cells were also found to be more inhibited by ethanol than growing cells. A maximum productivity of 27 g/L/h ethanol with a cell concentration of 85 g/L was projected for the system.

Damiano et al. (1985), using ultrafilters of 20,000 molecular weight cutoff (MWC) studied the effect of varying growth rate by changing the ratio of filtrate flowrate to total feedrate at a constant dilution rate of 0.2 h^{-1}. They found that the growth rate and specific ethanol production rate were inhibited to a greater extent than previously reported by Jin et al. (1981) using a CSTF and assumed the reason for this was the build-up of a toxin which could not pass through the membranes. The toxin production rate was thought to be proportional to the ethanol production rate. They then modelled the overall inhibition using an exponential model previously suggested by Aiba (1968), but with values of 0.404 h^{-1} and 0.59

h^{-1} for μ_m and ν_m and 0.075 g/L and 0.042 g/L for k_1 and k_2. As the bleed ratio (the ratio bleed to inlet flowrates) decreased, an increase in ethanol yield from 0.45 to 0.48 g/g and a drastic decrease in cell yield from 0.16 to 0.01 g/g was found.

Using a 50,000 MWC ultrafilter with total recycle, Lee and Chang (1987) found that growth stopped once an ethanol concentration of 90 g/L was reached. With a feed glucose concentration of 85 g/L, they also found that $Y_{x/s}$ and $Y_{p/s}$ equalled 0.136 g/g and 0.454 g/g respectively, while at a glucose concentration of 140 g/L, $Y_{x/s}$ decreased to 0.12 g/g, with both yields decreasing further at high cell concentrations. When the system was operated with a bleed and keeping the fermentor product and substrate concentrations at 45 g/L and 5 g/L respectively, they observed that both the growth rate, μ, and the specific ethanol production rate, ν, decreased with cell concentration. They modelled this in the following forms:*

$$\mu = 0.21 \qquad x \leq 40 g/L$$
$$\mu = 0.254\left(1.0 - \frac{x}{255}\right) \qquad x > 40 g/L \qquad (1)$$

$$\nu = 0.68\left(1.0 - \frac{x}{640}\right) \qquad (2)$$

*Note: All variables are defined in the Nomenclature section starting on page (x) at the start of this report.

Coppella and Dhurjati (1989), using a hollow-fibre bioreactor with a 10^6 MWC ultrafilter, total cell recycle and cell concentrations up to 35 g/L, found that the cell density had very little effect on yields or cell growth. They measured values for $Y_{x/s}$ and $Y_{p/s}$ of 0.15 and 0.49 g/g and for μ_m and K_s of 0.44 h^{-1} and 2.1 g/L, respectively.

Mota et al. (1987) used a carbon coated zirconium oxide membrane with a pore size of 0.14 μm to filter cell concentrations up to 160 g/L and obtained a maximum productivity of 80 g/L/h. They then modelled the specific ethanol production rate based on the following equation:

$$v = 0.74e^{-0.0045dx} \tag{3}$$

Using the same filter as Mota et al. (1987), Lafforgue et al. (1987), obtained cell concentrations of 300 g DW/L after 100 hours of total cell recycle. Beyond 75 g DW/L, they found that the viability of the cells started to decrease until it reached 75 %. At high cell concentrations, it was also found that the average size of the cells and the intracellular water content decreased.

Jarzebski et al. (1989) then used this data to model high yeast concentration fermentations, they suggest the following kinetics:

$$r_x = 0.24\left[1-\left(\frac{p}{90}\right)^{1.1}\right]\left[1-\left(\frac{x}{320}\right)^{1.1}\right]\frac{sx_v}{(0.5+s)} \tag{4}$$

$$r_d = 0.208(r_x+0.001x_v) \tag{5}$$

$$r_s = \frac{-r_x}{0.12}-0.27x_v \tag{6}$$

$$r_p = 0.55x_v e^{-0.0061x_v} \tag{7}$$

and used these equations in both an intrinsic (taking into account cell volume) and a non-intrinsic model. Although both models were found to fit Lafforgue et al.'s (1987) data fairly well, their models seem to be contradictory to many previous results. For instance, the equation for ethanol productivity is independent of both ethanol and glucose concentrations. It was also suggested by the authors that intrinsic kinetics were an unnecessary complication as the two models were very similar. This, however was based on the assumption that substrate and ethanol concentrations in the fermentor were the same as those after the cells had been removed. At high cell concentrations this is obviously not a good approximation.

3.3 Simulation and Optimization of a Cell Recycle Fermentor

The first published simulation of ethanol fermentation using cell recycling was given by Pollard (1981). The following steady-state non-intrinsic model was used -

$$p = \frac{(Bx - x_I)}{0.16} + p_I \tag{8}$$

$$s = \frac{(Bx - x_I)}{0.06} + s_I \tag{9}$$

$$\frac{f(x_I - Bx)}{V_F} = \frac{0.24\left(1 - \frac{p}{90}\right)\left(1 - \frac{x}{100}\right)sx}{(1.6+s)} \tag{10}$$

$$(r+e)x = rx_I \tag{11}$$

This model predicts that the dilution rate can be increased while giving the same product concentration if the bleed ratio is decreased. At an

inlet substrate concentration of 100 g/L, and with 98% substrate conversion, the model predicts that the relative productivity (the productivity of the fermentor divided by the productivity of a conventional CSTF operated at the same feed stream composition and conversion) increases as the bleed ratio, B, decreases to a value around 4 which occurs when the cell concentration is close to its maximum. Beyond this, a further decrease in bleed ratio and resultant increase in cell concentration causes a sharp decrease in productivity. On changing the maximum cell concentration and the cell concentration inhibition constant, only small changes to the model were found at bleed ratios above 0.1. However, Figure 14 of Pollard's thesis (1978) showed the value of these variables to be significant at low bleed ratios. The values of the kinetic parameters used in their work were not typical of the values used by most other researchers. The values for the growth rate, Monod constant and yields were obtained from experimental data using immobilized cells, where the kinetics of the cell are likely to be very different from the freely suspended case. For instance, the value of product yield calculated from their figures is 0.375 g/g, which is considerably lower than the typical values of between 0.45 and 0.5 g/g. The maximum cell concentration for growth also seems very low in light of Lee and Chang's (1987) data. Pollard bases this on Cysewski and Wilke's paper (1978), although in this reference, cell concentrations as high as 124 g/L D.W. were reported.

Monbouquette (1987) modelled cell recycle processes using the following intrinsic equations:

$$\frac{dX}{dT} = \frac{(1-P)SX}{(S+1)} - DBX \qquad (12)$$

$$\frac{dS}{dT}(1-X) = D(S_I-S) - \frac{(1-P)SX}{(S+1)}[\phi-S] \qquad (13)$$

$$\frac{dP}{dT}(1-X) = -DP + \frac{(1-P)SX}{(S+1)}[\pi+P] \qquad (14)$$

Fitting the model kinetic parameters to the experimental data given by Ghose and Tyagi (1979), the effect on the system's dynamics of increasing substrate concentration from 150 to 165 g/L was compared for intrinsic and non-intrinsic models. The difference between substrate concentrations in both these models becomes noticeable when the cell concentration reached 24 g DW/L, and increased until at cell concentrations of 96 g DW/L, a 15 % error in the culture substrate concentration was found. This error is equivalent to a 100% increase in the unconverted substrate fraction.

Using the kinetic equations given above, Jarzebski et al. (1989) also simulated steady state operation with a cell bleed. For a dilution rate of 0.5 h^{-1}, the maximum productivity predicted by the model was 33.2 g/L/h at a viable cell concentration of 164 g/L and a bleed ratio of 0.03.

An economic analysis based on the simulation of a variety of ethanol fermentation processes using molasses and cellulose hydrolysate as feedstocks was given by Maiorella et al. in 1984. Based on the prices given in Table 3 for mid 1981 and a plant producing 10^9 L/yr of 95 % wt. ethanol, Maiorella et al. (1984) used the following kinetics, based on data from Bazua and Wilke (1978):

$$v = 1.85\frac{s}{(0.315+s)}\left[1-\frac{p}{87.5}\right]^{0.36} \qquad (15)$$

$$Y_{X/P} = 0.249 \quad (16)$$

$$Y_{P/S} = 0.434 \quad (17)$$

Using molasses and assuming a fermentor sugar concentration of 2.8 g/L, they determined the fermentor conditions to give the lowest price to provide a 15 % after-tax return on total capital investment for each process as shown in Table 4.

Table 3. Raw material, utility and by-product costs used by Maiorella et al. (1984).

RAW MATERIAL COSTS ($)		UTILITY COSTS ($)		BY-PRODUCT CREDITS ($)	
MOLASSES (47.5 % UTILIZABLE SUGARS) /TONNE	84 -	41 BAR STEAM / TONNE	9.74	YEAST (50 WT% DRY) /TONNE	114
PROCESS WATER / 1000 M^3	133	3.4 BAR STEAM / TONNE	7.00	1 BAR STEAM / TONNE	1.69
NUTRIENTS / 1000 L ETHANOL	11.1	ELECTRICITY / MWh	88.2		
		COOLING WATER /1000 M^3	59.6		

Table 4. Optimized fermentor conditions and net costs given by Maiorella et al. (1984) for a variety of processes assuming a fermentor sugar concentration of 2.8 g/L.

PROCESS	FERMENTOR CONDITIONS (g/L)			NET COST
	FEED SUGAR CONCN.	CELL CONCN.*	ETHANOL CONCN.*	($US/ 1000 L)
BATCH	167	INITIAL 2.1 FINAL 21.3	INITIAL 8.6 FINAL 85.6	530.5
CSTF	156	19.7	79.1	512.9
RECYCLE CSTF	167	100	85.6	490.6
PARTIAL RECYCLE CSTF	156	50	79.1	497.6
2 CSTF IN SERIES	167	FIRST 17.4 SECOND 21.3	FIRST 70.0 SECOND 85.6	509.6
PERFORATED PLATE COLUMN	169	OUTLET 21.6	OUTLET 86.9	523.4
PLUG FLOW	167	INLET 36.0 OUTLET 53.0	INLET 17.1 OUTLET 85.6	491.1
TOWER FERM.	167	70.0	OUTLET 85.6	487.1
EXTRACTIVE	384	100	80.0	439.4

*Values are in g/L Water.

They concluded that high cell density fermentations allow the fermentation costs to be reduced as high fermentor productivities can be maintained at high ethanol concentrations. Selective ethanol removal using a process such as extractive fermentation could offer even greater savings as even more concentrated feeds can be used.

4 THEORY

A CSTF with cell recycle has two characteristic parts: the fermentor and a cell separating device. To obtain a steady state when using cell recycle with growing cells, a cell bleed stream is necessary to keep the cell concentration constant. This is known as partial cell recycle, while if the system is run without a bleed, it is known as total cell recycle. Two basic variants of partial cell recycle are possible: internal bleed, where the bleed stream is upstream from the cell concentrating device; and external bleed, where the bleed stream is downstream from the cell concentrating device. (Note that Monbouquette (1987) has previously called these internal and external recycle respectively). The difference between these two designs is that in the first case the cells are taken off at the same concentration as in the fermentor, while in the second case, the bleed cell concentration is higher than in the fermentor.

4.1 Cell Recycle Kinetics

The kinetics of a recycle system, especially one using cross-flow filters, should be similar to that of a CSTF system, as over 90 % of the fermentation is taking place in the fermentor and the residence time in the filter is comparatively small. However there are two factors in which the system differs; firstly a much higher cell concentration exists and secondly there are two separate product streams from the fermentor system.

High cell concentrations make use of 'intrinsic kinetics', where the volume of the cell is, much more important than at lower cell concentrations and so must be taken in account. Another possible effect

of high cell concentrations is a decrease in growth rate due to mass transfer limitations. At high cell concentrations, the cells are in much more immediate contact with each other, so there is less surface area for the nutrients to enter the cell and more resistance may occur due to larger cell aggregates. This is one reason why the cell inhibition suggested by Lee and Chang (1987) may exist.

Some larger molecules may also not pass through the filter and, especially if there is no bleed to remove these molecules, inhibitory concentrations may be reached. The concentration of such inhibitors may vary substantially depending on the bleed ratio and the type of filter used. Their effects on the cells may also be dependent on the concentrations of nutrients present in the fermentor. The decrease in growth rate with increasing cell concentration found by Lee and Chang (1987) could just as easily have been due to the accompanying decrease in bleed ratio and increase in inhibitor concentration.

A further complication of a cell recycle system compared to a CSTF, as found by Lafforgue et al. (1987), is that the percentage viability can decrease significantly as the cell concentration increases. However, their results were obtained using a cell recycle system with no bleed. As has been previously stated by Cheryan and Mehaia (1986), a cell bleed is necessary to remove dead or inactive cells and macromolecular products of metabolism which do not permeate the membrane. When a sufficient bleed was used, Cysewski and Wilke (1977) found that no change in cell viability occurred over 14 days of continuous operation.

The yields for the kinetics were based on abiotic phase volumes, assuming that the fraction of the volume occupied by the cells is

proportional to their density, so that:

$$Y_{X/S} = \frac{X}{S_F - S(1-BX)} \tag{18}$$

$$Y_{X/P} = \frac{X}{P(1-BX) - P_F} \tag{19}$$

$$Y_{P/S} = \frac{P(1-BX) - P_F}{S_F - S(1-BX)} \tag{20}$$

The kinetic equations used in the fermentor model were taken from Warren et al. (1989):

$$\mu = \mu_m \frac{S}{(K_s+S)}\left[1-\left(\frac{P}{P_{m1}}\right)^a\right] \tag{21}$$

$$Y_{X/S} = (Y_{X/S})_0\left(1-\frac{P}{P_{m2}}\right) = \frac{\mu}{q} \tag{22}$$

$$Y_{X/P} = (Y_{X/P})_0\left(1-\frac{P}{P_{m3}}\right) = \frac{\mu}{\nu} \tag{23}$$

These bio-kinetic equations were based on data for a CSTF with exogenous ethanol and as such, modifications for cell inhibition and cell death may need to be added.

4.2 Fermentor Simulation

The model is derived for a CSTF with external cell recycle but is easily modified to the internal cell recycle case. As well as using the yield equations (eqns. (18) to (20)) and kinetic equations (eqns. (21) to (23)) above, the simulation has the following assumptions: i) the

fermentor is perfectly mixed and has a constant volume and temperature, ii) the volumetric flow rate in and out of the fermentor are the same, and iii) no solids pass through the filter so that there is a perfect separation.

The model is based on five component balances: i) total flow over the system (eqn. 24), ii) biomass over the system (eqn. 25), iii) biomass over the cell separator (eqn. 26), iv) substrate over the system (eqn. 27) and v) product over the system (eqn. 28):

$$f_F = b + e \tag{24}$$

$$\frac{d(xV_F)}{dt} = -bx_b + [1-(p/p_{m1})^a] \frac{\mu_m s x V_F}{(K_s+s)} \tag{25}$$

$$(r+f_F)x = (r+b)x_b \tag{26}$$

$$\frac{d[(1-x/\rho)sV_F]}{dt} = f_F s_I - bs(1-x/\rho) - es - \frac{[1-(p/p_{m1})^a]\mu_m s x V_F}{(Y_{x/s})_0 (1-(p/p_{m2})(K_s+s)} \tag{27}$$

$$\frac{d[(1-x/\rho)pV_F]}{dt} = f_F p_I - bp(1-x/\rho) - ep + \frac{[1-(p/p_{m1})^a]\mu_m s x V_F}{(Y_{x/p})_0 (1-(p/p_{m3})(K_s+s)} \tag{28}$$

In dimensionless form these become:

$$B + E = 1 \tag{29}$$

$$\frac{dX}{dT} = -DBXk + \frac{(1-P^a)SX}{(S+1)} \tag{30}$$

There are now five equations with ten variables, so five variables need to

$$\kappa = \frac{(R+1)}{(R+B)} \tag{31}$$

$$\frac{dS}{dT}(1-X) = D(S_I-S) - \frac{(1-P^a)SX}{(S+1)}\left[\frac{\phi_0}{(1-K_2P)} - S\right] \tag{32}$$

$$\frac{dP}{dT}(1-X) = D(P_I-P) + \frac{(1-P^a)SX}{(S+1)}\left[\frac{\pi_0}{(1-K_3P)} + P\right] \tag{33}$$

be specified. If these are taken as P_I, S_I, S, R and B, then at steady state:

$$\begin{aligned} f(P) = {} & S_{IK_2}K_3P^3 + [\phi_0K_3 - (K_2+K_3)S_I - K_2K_3SP_I]P^2 + \\ & [(S-S_I)\pi_0K_2 - \phi_0(P_IK_3+1) + P_IS(K_2+K_3) + S_I)]P + \\ & (S_I-S)\pi_O + P_I(\phi_0-S) = 0 \end{aligned} \tag{34}$$

$$D = \frac{S(1-P^a)}{B\kappa(S+1)} \tag{35}$$

$$X = \frac{(S_I-S)(1-K_2P)}{B\kappa[\phi_0-S(1-K_2P)]} \tag{36}$$

where eqn. (34) can be solved by Newton-Raphson method (Riggs, 1988). This approach requires an initial guess of product concentration which is taken as the value produced if yields had remained constant.

$$P_{GUESS} = P_F + \frac{\pi_0(S_F-S)}{\phi_0} \tag{37}$$

To account for cell inhibition of the growth rate, the only change necessary occurs in eqn. (35), which then becomes:

$$D = \frac{S(1-P^a)(1-X)}{B\kappa(S+1)} \tag{38}$$

Changes in cell viability can be taken into account by adding a term for

the fraction of viable cells, V_b, to eqn (36), which then becomes:

$$D = \frac{S(1-P^a)\,V_b}{B\kappa(S+1)} \tag{39}$$

To use these equations for a CSTF with internal bleed, κ is set equal to 1, while to solve for a CSTF with no cell recycle, both κ and B are set equal to 1.

4.3 Economic Optimization of the Cell Recycle System

To be able to economically optimize the cell recycle system, prices for the streams entering and leaving the system are required. The cost of these streams is dependent on their concentrations, so to obtain a true optimization, all of the plant would need to be simulated. However, the optimization of the whole plant was out of the scope of this project. Instead, simple cost equations were used to take into account the effect of different cell recycle inlet and outlet streams.

For the fermentor simulation, the intrinsic model previously presented (eqns. (34) to (36)), was used. The effects of scale-up were assumed to be negligible as the membrane surface area can be increased by increasing the number of tubes. Because of the much smaller residence time, no reaction was assumed to occur in the filter or the tubing connecting it to the fermentor. This is obviously an approximation, but considering the other uncertainties in the model, a justifiable one. The model for the membrane filter was taken from Warren et al. (1991):

$$j_{AV} = \frac{K_3\left((P_I+P_O)/2 - P_E\right)^{1-K_{M1}}}{\gamma_I^{K_{M2}} X_m^{K_{M3}}} \tag{40}$$

$$(P_I - P_O) = K_4 \gamma^{K_{M4}} (1 + K_{M5} + X_M)^2 \quad (41)$$

This was then adapted to take into account the number of tubes and the change in units to the following equation:

$$F_M = \frac{0.108\left[P_{MI} - 0.054\left(\frac{F_{CR}}{A}\right)^{1.2}(1+0.0043X_M)^2\right]^{0.24} F^{0.1}A^{0.9}}{X^{0.45}} \quad (42)$$

The cost functions per hour (eqns. (43) to (46)) were based on: a) the grain and the stages upstream of the fermentor, b) the fermentor, c) the membrane filter, and d) the stages downstream of the filter. The sum of these cost functions minus the credit for the by-products then gives the production cost/h, from which a cost/1000 L can be obtained.

$$C_{SUBS} = C_{S1} S_F F_F \quad (43)$$

$$C_{FERM} = C_{P1} (V_F)^{0.7} \quad (44)$$

$$C_{MEMB} = C_{M1} A^{0.7} + C_{M2} A^{0.8} + C_{M3} P_I F_{CR} \quad (45)$$

$$C_{DIST} = (C_{D0} + C_{D1} P + C_{D2} P^2) F_{PR} + C_{D4}\left(\frac{Y_{X/P}}{X}\right)^{0.7} + C_{D5}(Y_{Y/P})^{0.7} + C_{D6} Y_{X/P} \quad (46)$$

The cost/h for the grain and those stages upstream from the fermentor was calculated from the capital and utility costs directly related to it and a third of the personnel costs and was assumed to be approximately constant with substrate concentration. An assumption was made that the flowrate of backset going directly to the fermentor is set by a limiting concentration of unfermented solids rather than as just a fraction of the new water added. As such, the amount of new water added and the starch concentration entering the cooker do not vary when the

inlet substrate concentration changes, so the variation in cost with substrate concentration is negligible. The cost/h of the fermentor was calculated using a size exponent of 0.7 as given by Kalk (1986). The cost of the membrane itself was calculated based on a size exponent of 0.8, similar to that shown graphically by van Gassel and Ripperger (1985) and a size exponent of 0.7 was used for the supporting equipment. As costs for the hollow-fibre microfilter required here were not available, the pricing was based on values given by Tutunjian (1983). It was also assumed that the membrane would need to be replaced every two years. The cost/h of the downstream stages was calculated based on the capital and utility costs directly related to it and two-thirds of the personnel costs. As the ethanol concentration fed to the distillation column varies, significant changes occur in the size of the first distillation column and in the energy used in this column. Figures showing how these vary are given graphically by Zacchi and Axelsson (1989). From these figures, a second order correlation for cost with respect to the inlet ethanol concentration was derived. The cost of separating and drying the yeast depend on the size and concentration of the yeast in the stream. This was modelled using a constant proportional to the amount of yeast for steam costs, and with a size exponent of 0.7 used for drying, cooling and centrifugation capital costs. The value of the yeast by-product was based on its protein content compared to dried grains. Depending on the site, a higher value for the product may be possible when used other than as a cattle protein feed.

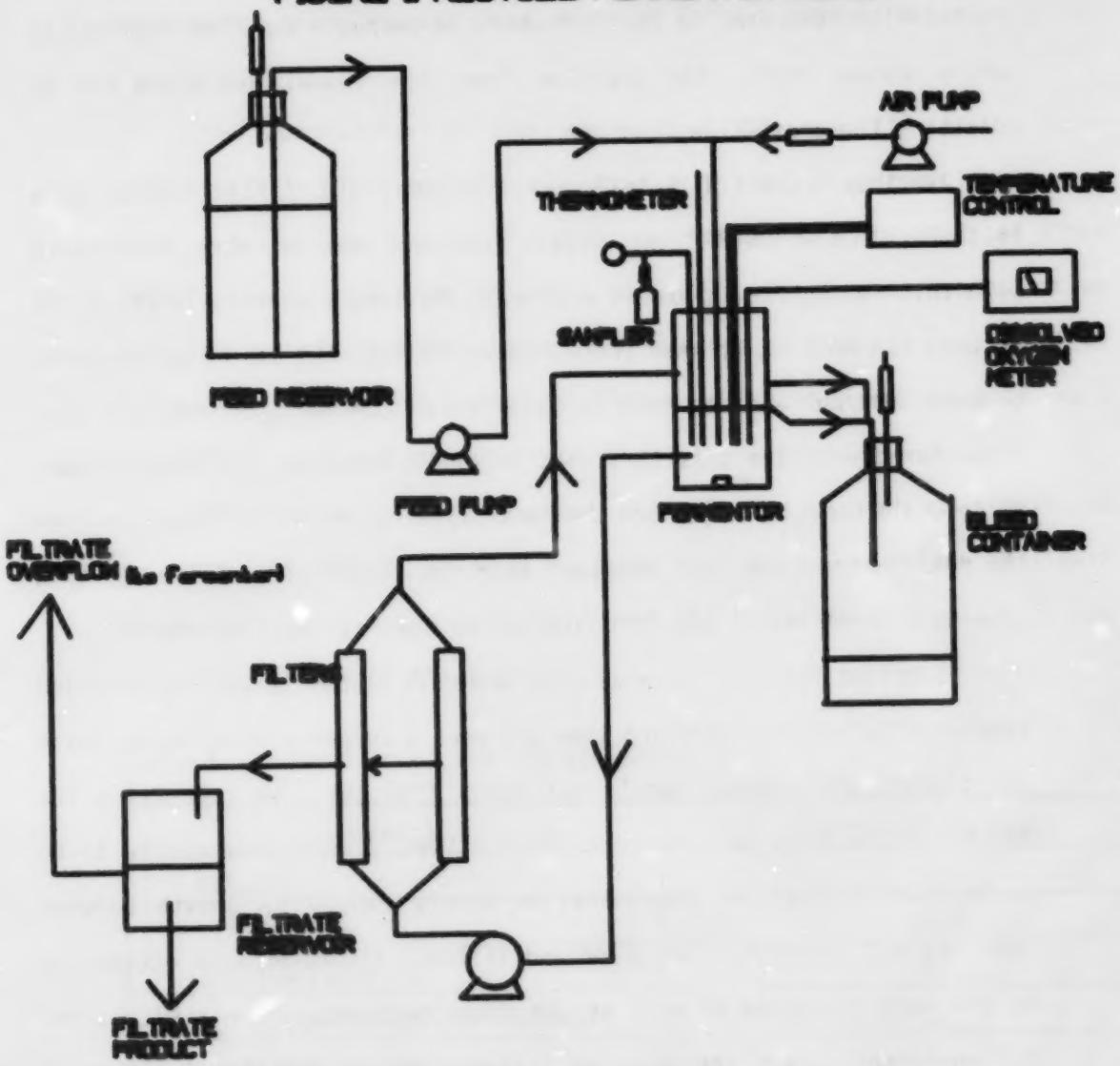
5. METHODS

5.1 Experimental Equipment

A general sketch of the final apparatus used is shown in Figure 1, from which it can be seen that the system was operated with an internal bleed. A one litre New Brunswick (Edison, NJ) Bioflo C32 fermentor was adapted to give a 500 mL working volume by adding an extra tap as an overflow line. Because of problems with yeast caking on the rod immersion heater, an external heater tape wrapped around the outside of the fermentor was used for heating. The stirrer speed was set at 400 RPM., as at lower speeds problems with viability occurred due to poor mixing. The temperature was set at 30° C. It was first thought that some form of cooling would be required, especially for high cell concentrations. This was not found to be true, so the cooling finger used in initial experiments was removed. An aeration rate of 0.4 L/min was used, with sterilized air entering through sparging holes in the stirrer and through the feed tube. Further details on the fermentor are given in the New Brunswick Scientific manual (1989).

A 20 L Pyrex carboy was used for the feed reservoir and 10 L plastic containers were used for the filtrate and bleed containers. The cross-flow membranes consisted of approximately 80 polysulphone hollow fibres and were produced by A/G Technology (Needham, MA). The tubes were 0.75 mm ID and had a nominal pore size of 0.2 μm. This type of filter is backflushable and autoclavable. Initially one filter was used, but, because of overnight problems and as the final runs needed high filtrate flows, two and later three filters connected in parallel were used. Because gas bubbles were able to leak through the filter, a constant

FIGURE 1. RECYCLE FERMENTOR SETUP



filtrate flux from the filters was impossible to achieve. A filtrate reservoir was then added so that a constant filtrate flowrate could be removed from the system. A 350 mL Pyrex vessel was used for this, with the overflow returning to the fermentor. To decrease the time required to obtain steady state, the overflow from this vessel was minimized by adjusting the cross-flow flowrate.

Various sizes of Cole-Parmer (Chigaco, IL) C-flex tubing were initially used to connect the stages. Problems, however, were encountered with this tubing due to excess wear over the long timescale in which the system was operated. Pharmed (Cole-Parmer, Chicago IL) tubing was found to be much superior and was used in later runs. Further problems were also encountered with the size 13 tubing initially used for the feed stream, which would occasionally block due to a small number of particulates from the yeast extract and the antifoam. With the larger size 14 tubing, this problem did not occur, but it could not be used at low flowrates.

Sampling for cell concentration and cell viability was via a hooded sampler attached to a sampling tube and used a rubber suction bulb, while the glucose and ethanol sample was taken from the line connecting the filter to the filtrate reservoir. This allowed a cell-free sample to be taken, thus minimizing concentration errors caused by growth between sampling and filtering. The bleed and filtrate flowrates were determined by the rate of change of mass of the containers and contents with time. All equipment except the pressure transducers was sterilized either at 121°C for 25 minutes or 135°C for 5 minutes. The pressure transducers were sterilized using 70 % ethanol.

5.2 Organism and Media

Saccharomyces cerevisiae NRRL Y-132, obtained from Northern Regional Research Laboratories (Peoria, IL), was used for all experiments. The pure culture for inoculums was grown on Yeast Extract-Peptone-Dextrose (YEPD) Agar kept at 4°C.

The composition of the media used for the 150 g/L glucose experiments is given in Table 1. Citric acid and tri-sodium citrate were used to buffer the medium. The exact concentrations were adjusted after autoclaving to give a pH between 3.95 - 4.05. The glucose concentration was also tested after autoclaving and adjusted so that the required feed value was obtained. The commercial glucose source used was hydrated, so a 10 % higher mass was usually required.

To minimize Grignard reactions, the glucose solution was sterilized separately at 121 °C for 30 mins. Because of the large volume of liquid being sterilized, a one and a half hour sterilization time at 121 °C was used for the rest of the media.

Table 5. Composition of the 150 g/L media.

CHEMICAL	CONCENTRATION (g/L)
GLUCOSE	150
YEAST EXTRACT	15.0
CALCIUM CHLORIDE	0.1
POTASSIUM DIHYDROGEN PHOSPHATE	2.1
MAGNESIUM SULPHATE	0.5
SODIUM CITRATE	5.5
CITRIC ACID	5.5
DOW ANTIFOAM B	0.5

5.3 System Operation

The primary aim of these experiments was to obtain steady state data, so that conditions were kept as constant as possible until steady state was obtained. The following set of operations were performed to obtain steady state results.

i) The apparatus and media were sterilized and the apparatus was then aseptically connected together.

ii) An inoculum solution was grown in a 250 mL Erlenmeyer for 2 days using yeast from a pure culture YEPD slant. The media of the same strength as the experiment was used, with the yeast extract and minerals and glucose having been sterilized separately.

iii) A sample was then taken from this inoculum solution to check for viability and contamination. If the inoculum was not contaminated and had at least a 75% viability, 10 mL was then added to the fermentor. The overflow line at 500 mL was clamped off and sufficient feed to fill the fermentor (990 mL) was added. The stirrer, temperature controller and the air pump were then activated.

iv) After 24 hours of batch operation, the optical density (O.D.) was checked to see if it was sufficiently high for the system to be started in continuous mode. The filter pump was first turned on and the filtrate reservoir was then allowed to fill. Once this had occurred the feed and filtrate pump were turned on and adjusted so that the fermentor volume became 500 mL. The clamp from the lower bleed/overflow line was then removed and the feed and filtrate flowrates were set for the conditions to be measured.

v) Regular samples (once every two to three hours) were then taken until

the system reached steady state. A 5 mL sample was taken for cell concentration and viability analysis, with a one to two mL sample taken for ethanol and glucose analysis and then frozen. As the cell concentration was often found to be constant before the glucose and ethanol concentrations, steady state was assumed to have occurred when the cell concentration was constant for at least three measurements and the glucose concentration was constant at least twice.

vi) Once steady state was obtained, the experimental conditions were changed to that of the next data point and step v) repeated.

5.4 Filter Backflushing

Backflushing of the filter was found to be required approximately once every 24 hours as otherwise the filtrate flowrate significantly decreased. It allowed some of the fouling to be washed away, thereby giving higher fluxes. The following is an outline of the method used:

i) Turn off the filtrate, filter and feed pumps.

ii) Close the bleed line and the line connecting the fermentor to the filtrate reservoir.

iii) Put the filter pump in reverse and slowly increase flow to 250 mL/min. Watch for five minutes.

iv) Slow the filter pump to 10 mL/min and close both the fermentor-filter tubing at the end not connected to the pump and the filter-filtrate reservoir tubing nearest to the pump end of the filter. Watch for ten minutes

v) Turn off the filter pump and remove all the clamps except on the bleed line. Take the tubing out of the filter pump and replace it with the tubing from the other end of the filter.

vi) Turn the filter and feed pumps on and readjust them to their original flows. When the filter reservoir has returned to its original level, turn the filtrate pump back on and remove the clamp from the bleed line.

5.5 Analytical Methods

The following variables were measured: i) pH, ii) cell concentration, iii) ethanol concentration, iv) glucose concentration and v) methylene blue viable count. The pH and glucose concentrations were also measured for the feed. The pressures were determined using Validyne (Northridge, CA) transducers and demodulators.

The variables were measured as follows:

i) pH was measured by a Chemcadet (Cole-Parmer, IL) Series 5986 pH meter.

ii) The cell concentration was measured using a Pharmacia (Cambridge, U.K.) Novaspec II Spectrophotometer, by measuring adsorption at 600 nm. This was then compared to a standard curve of adsorbance against cell dry weight. The cell weight was previously determined by drying in a Blue M (Blue Island, IL) StabilTherm oven, with the sample being left overnight at 105 °C. Before drying the cells were centrifuged three times at 5000 RPM for fifteen minutes, with the liquid being decanted off and replaced by water each time.

iii) The ethanol concentration was measured using a Hewlett-Packard (Palo Alto, CA) 5890 gas chromatograph (G.C.) with a flame ionization detector. The temperatures used were: oven 60°C, injection 200°C and detector 230°C, while the column pressure was 25 psig. Nitrogen was used as the make-up gas, and Helium as the carrier gas. One mL of an ethanol standard close

to the ethanol concentrations expected from the run was first added to one mL of 45 g/L iso-butanol, which was used as an internal standard. After washing the needle, three 1 μL injections were made into the G.C., two minutes apart. The average of the ethanol to butanol peak ratios for the three injections was then recorded. One mL of each experimental sample and one mL of the internal standard were then mixed and three 1 μL injections ywere made as for the ethanol standard. The sample at the highest dilution rate was taken as the first sample and the lowest dilution rate as the last sample.

iv) The glucose concentration was measured by a Yellow Springs Instrument (Yellow Springs, OH) 23A glucose analyzer. The sample was first diluted so its glucose concentration was between 1 and 5 g/L and then injected into the analyzer. For further details on the analyzer see the YSI 23A analyzer instruction manual (YSI Inc., 1989).

v) For the methylene blue viable cell count, the sample was diluted with sufficient sterile 0.1 % peptone solution to give between 50 - 200 cells per field and enough 0.1 % methylene blue so that the cells were sufficiently stained. 0.1 mL of this mixture was placed on a clean microscope slide. After ten minutes, the total number of cells and those still stained blue were counted for at least 500 cells using a Baush and Lomb (Rochester NY) photo-zoom inverted microscope. The cells which were stained blue were non-viable, from which a percentage viability was obtained.

5.6 Fermentor Model Parameter Estimation

The model parameters were calculated using a commercial, non-linear,

multiple regression technique based on the Levenburg-Marquardt algorithm (UNLSF marketed by IMSL, Houston TX). Values of standard errors for the model parameters were then calculated using Jacobians at the least squares locations.

5.7 Simulation, Costing and Optimization

A Turbo-Pascal program was written using the equations and cost functions given in the theory section to find the cost of each stage at various combinations of concentrations and flowrates. The cost of raw materials, utilites and by-product credits is given in Table 6. Table 6 also shows the assumed conversions for stages other than the fermentor.

Table 6. Important Raw Material, Energy and Product Costs and Yields used in the Simulation and Optimization of a 10^8 L 99.2 % w/v Ethanol Production Facility (values are per unit of raw material).

RAW MATERIAL	COST $CAN	UTILITY	COST $CAN	PRODUCT	COST $CAN
WHEAT† /T (62.6 % STARCH)	90	NATURAL GAS† /1000 M^3	100	ETHANOL‡ /1000 L	460
GELATINIZATION ENZYMES /T	5000	ELECTRICITY† /GWh	250	DRIED GRAINS† /T	180
AMMONIA /T	250	WATER† /1000 M^3	4	DRIED YEAST★ /T	700

STAGE	COOKING	SACCHARIFICATION	OVERALL
PERCENTAGE YIELD BY WEIGHT	98 STARCH TO DEXTRINS	97 DEXTRINS TO UTILIZABLE SUGARS	28 WHEAT TO DRIED GRAINS

† Costs taken From values for Saskatoon
‡ Anonymous (1991a)
★ Anonymous (1991b)

The next step was to find the best operating conditions to minimize the overall production costs. This was achieved by incorporating an optimization strategy into the program. Fortunately, this problem can be

simplified as three of the variables, the inlet membrane filter pressure, the filter cross-flow flowrate and the filter area are important only in the filter. So, if the other variables in the filter are held constant, the filter can be optimized analytically using the method of Lagrange multipliers. This gives a set of simultaneous non-linear equations which can then be solved numerically using Newton's method. For the filter modelled here, the inlet pressure must be less than one bar. When optimized, this was found to be the optimum pressure. In the optimization routine this was taken as the inlet pressure to the filter.

The remaining independent variables (cell concentration, inlet substrate concentration and fermentor substrate concentration) were then optimized numerically using a variant of the steepest gradient method. The method used differs from the steepest gradient method in that the constant to determine the change in each variable from the partial derivative of the cost function, depends on the value of each variable. This change was made because the steepest gradient method becomes very slow as the correct solution is approached. Changing these constants allowed a faster approach to the correct solution. The problem is also constrained, although the steepest gradient is an unconstrained optimization technique. By varying the constants it is also possible to make sure the problem stays within the constraints.

6. RESULTS & DISCUSSION

6.1 Cell Recycle Fermentor Kinetics

The experimental results from the cell recycle experiments are listed in Table 7. It can be seen that cell recycle allows significantly higher productivities than a straight CSTF at similar conversions and product concentrations. The highest productivity obtained was 69.6 g/L/h, compared to a typical CSTF productivity with a similar product concentration of only 5 g/L/h. A graph of growth rates at d=0.34 h^{-1} versus ethanol concentration is shown in Figure 2. A decrease in growth rate with cell concentration did not occur, indicating that the cell inhibition previously found by Lee and Chang (1987) was not present. From the cell recycle results and sixteen previous results obtained from a continuous stirred tank fermentor with no recycle, the following best fit equation for growth rate was found:

$$\mu = 0.45\frac{s}{0.48+s)}\left[1-\left(\frac{p}{103.7}\right)^{0.65}\right]V_b \qquad (47)$$

A parity plot of the experimental against theoretical growth rates for the cell recycle results is given in Figure 3. When linear product inhibition was used, the following best fit values were found:

$$\mu = 0.38\frac{s}{(0.46+s)}\left(1-\frac{p}{94.5}\right)V_b \qquad (48)$$

Only a small increase in average error between the non-linear and linear equations was found (6.3 to 6.6%). This indicates that the biokinetic model is not sensitive to the exact value of the non-linear exponent. Using the same organism, a previous best fit of Ghose and Tyagi's data (Warren et al., 1990), found a value of 1.11 for 'a', while for the same data, Luong (1985) suggested a value of 0.8 .

Table 7 Experimental Results from the Cell Recycle Experiments

NO.	DILUTION RATE (h^{-1})	GROWTH RATE (h^{-1})	BLEED RATIO	CELL CONCENTRATION (g/L)	ETHANOL CONCENTRATION (g/L)	ETHANOL PRODUCTIVITY (g/L/h)
1	0.037	0.037	1.0	18.5		
2	0.072	0.072	1.0	17.1	69.8	5.0
3	0.10	0.10	1.0	12.8	66.6	6.7
4	0.34	0.04	0.11	83.2	70.2	23.9
5	0.34	0.06	0.19	40.6	69.1	24.1
6	0.33	0.09	0.28	32.0	68.9	22.7
7	0.34	0.16	0.47	16.4	46.8	16.0
8	0.58	0.06	0.11	67.2	69.8	40.8
9	0.58	0.08	0.14	54.1	69.4	40.5
10	0.58	0.11	0.19	43.0	66.1	38.3
11	0.58	0.17	0.29	22.3	50.1	29.1
12	1.02	0.11	0.11	82.0	67.0	68.3
13	1.00	0.06	0.06	117.7	69.9	69.6

Table 7 cond.

NO.	GLUCOSE CONCENTRATION (g/L)	CELL VIABILITY (%)	pH	CELL YIELD (g/g)	PRODUCT YIELD (g/g)
1	0.4	95.6	4.08	0.124	0.44
2	1.3	95.2	4.07	0.115	0.44
3	2.5	96.8	3.99	0.087	0.43
4	0.4	79.7	4.18	0.060	0.46
5	1.3	92.5	3.96	0.051	0.45
6	4.4	94.9	3.97	0.062	0.46
7	51.6	93.5	3.96	0.077	0.46
8	1.5	88.9	4.17	0.048	0.46
9	2.6	96.2	4.04	0.052	0.46
10	10.2	97.2	4.04	0.057	0.46
11	44.1	97.2	4.00	0.061	0.46
12	10.1	87.3	4.04	0.062	0.46
13	1.5	86.8	4.20	0.051	0.46

FIGURE 2. GROWTH RATE EFFECTS FOR A RECYCLE FERMENTOR
(Dilution Rate = 0.34 h^{-1}, S_0 = 150 g/L)

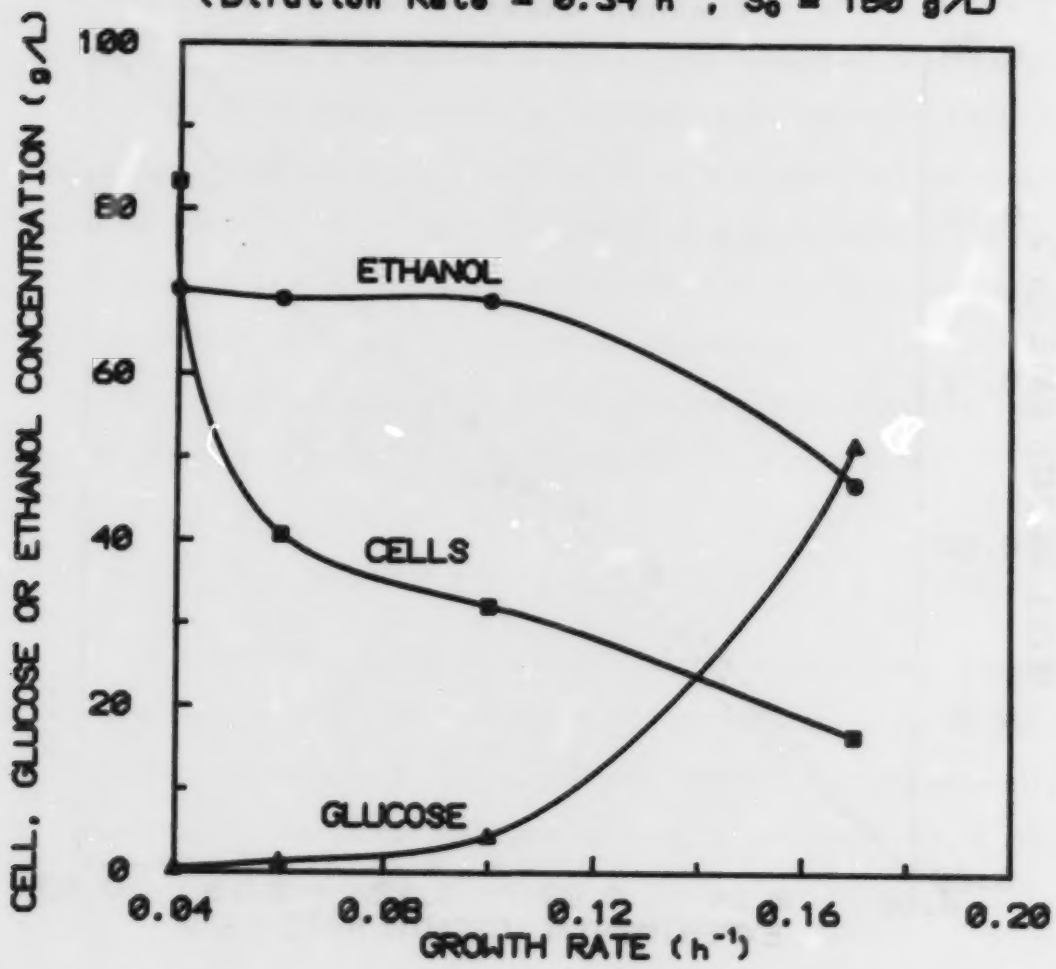
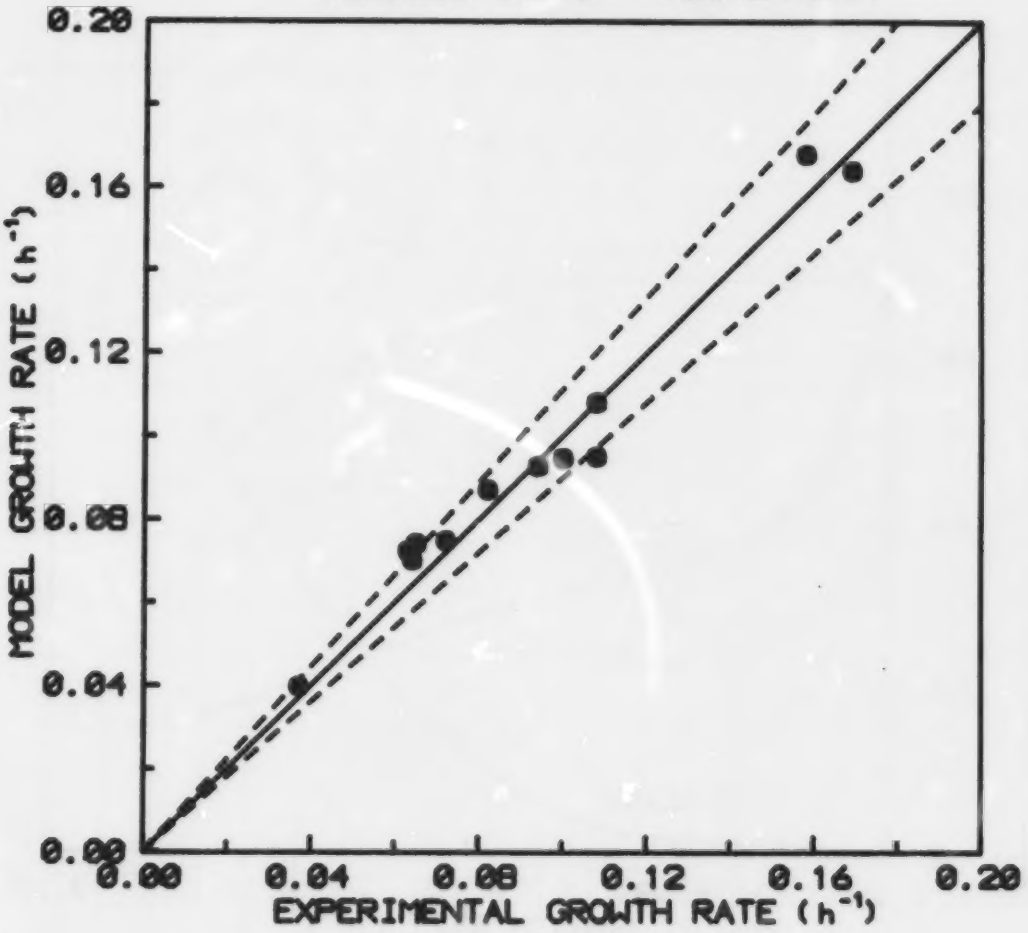


FIGURE 3. PARITY PLOT OF GROWTH RATE
(Dashed Lines = 10% Error).



At some of the higher cell concentrations, the cell viability decreased, with the lowest value being 80%. This decrease in viability, however, did not correlate directly with cell concentration but seemed to be more dependent on growth rates, with low growth rates giving lower viabilities. However, for the CSTF runs with no recycle, no decrease in viability occurred with low growth rates. The effect is therefore not simply from cells growing slowly. For recycle fermentation at low growth rates, there are also accompanying low bleed ratios and this is the likely cause of the decrease in viability. This is because, as was mentioned above, low bleed ratios may cause the build-up of larger toxic molecules which cannot pass through the membrane. Unfortunately there are only four experimental points where the decrease in viability was observed. This makes it difficult to develop an accurate model for viability, but the four points were best fit to the following linear relation:

$$\begin{aligned} V_b &= 0.96 && B > 0.15 \\ V_b &= 0.96[1 - 1.63(0.15 - B)] && B < 0.15 \end{aligned} \qquad (49)$$

The previous kinetics for a CSTF (Warren et al., 1990) found that the cell and product yields decreased with product concentration and that this decrease could be fit using a linear decrease of yield with ethanol concentration. When these equations were fit to the data above, decreases in both yields were again observed:

$$Y_{P/S} = 0.453\left(1 - \frac{P}{3060}\right) \qquad (50)$$

$$Y_{X/S} = 0.082\left(1 - \frac{P}{240.5}\right) \qquad (51)$$

It is clear the decrease in product yield with product concentration is

almost negligible (less than 3% over the whole range) so that only a small error would be introduced if it is assumed constant. Although this equation gives a reasonable fit for the cell recycle data, the physiology of how the cell yield changes is very complicated and more results are required before a more accurate model can be derived. The experimental and theoretically predicted cell yields are given in Figure 4.

One unexpected result, shown in Figure 5, is an increase in pH with cell concentration. The exact reason for this is unknown, but could perhaps be due to the buffering effect provided by the cells themselves. At higher cell concentrations, a considerable amount of the fermentor volume is occupied with biomass which likely ties up many free hydronium ions.

The greatest cell concentration used in these experiments was 120 g/L DW, although previous cell recycle results have shown that cell concentrations up to 300 g/L can be achieved (Lafforgue et al., 1987). At 120 g/L, considerable difficulty was found operating the fermentor due to foaming. Foaming was more of a problem than at lower cell concentrations because of the high fluid viscosity, so that the gas bubbles had problems escaping from the fluid. At any higher cell concentration, a different bioreactor design which would allow the escape of gas bubbles would be recommended. However, it is shown in the economic optimization analysis that because the flux in the membrane drops off fairly significantly with cell concentration, optimal cell concentrations are in the range of 50 - 100 g/L DW, so that this is not a real operating problem.

The membrane filters used had an ID of 0.75 mm and, as such, fouled very easily, especially at high cell concentrations. As well as causing

FIGURE 4. EFFECT OF ETHANOL CONCENTRATION ON YIELDS

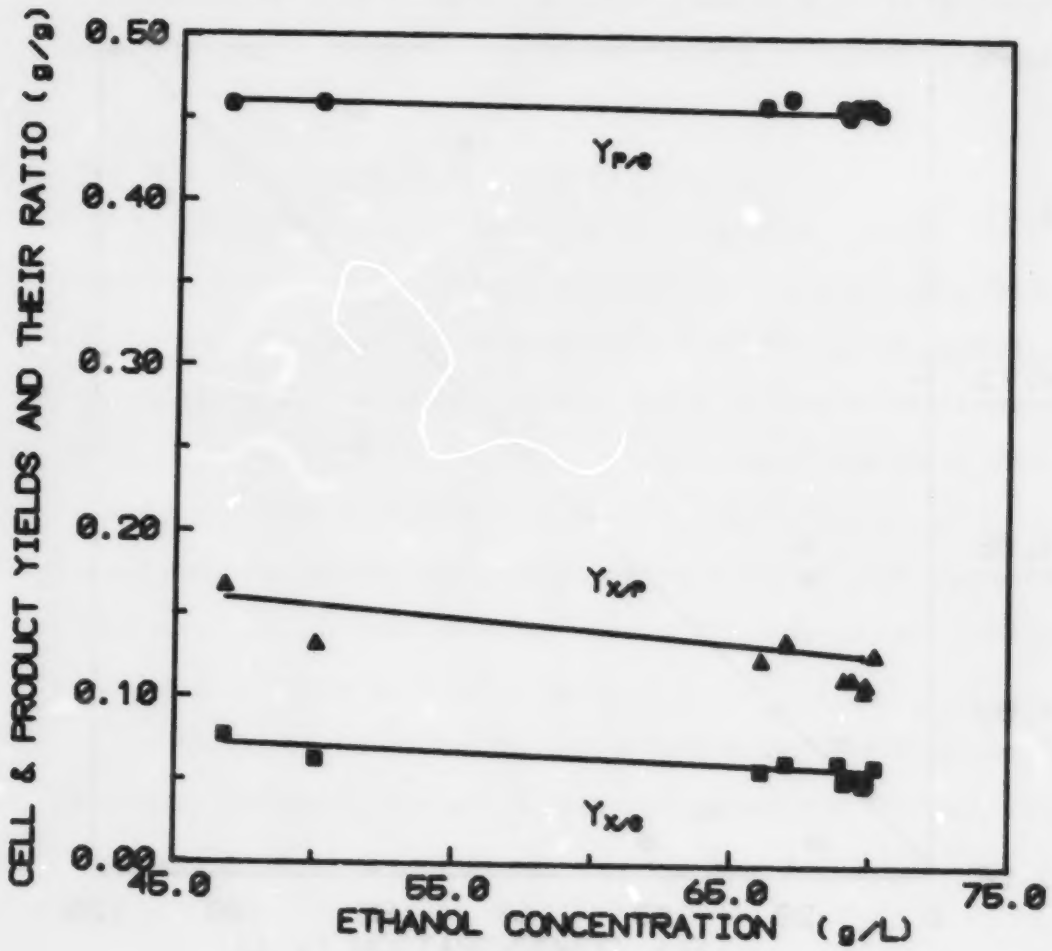
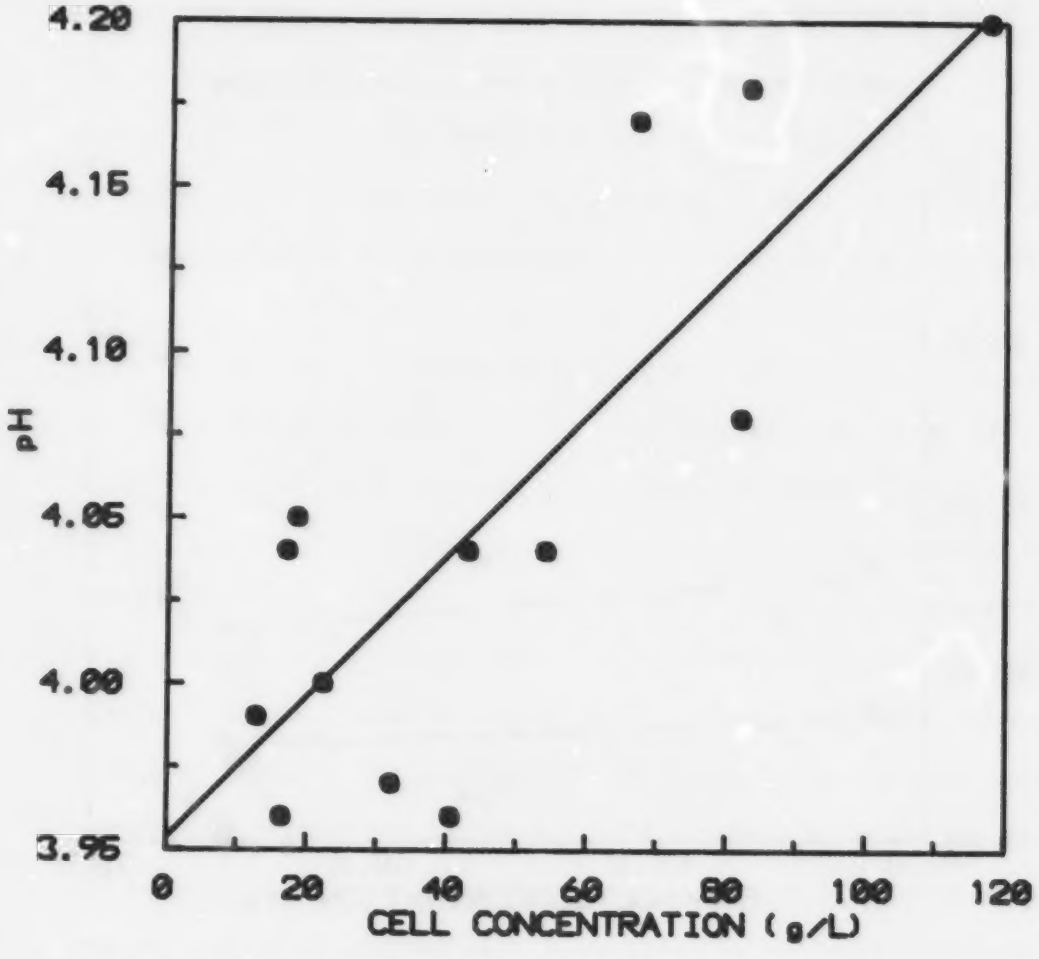


FIGURE 5. EFFECT OF FERMENTOR CELL CONCENTRATION ON pH.



low fluxes, this problem also meant that the filter had to cleaned by backflushing approximately once per day. Towards the end of the experiments, a 1 mm ID filter was used and an improvement in flux and reduced plugging was noticed. However, the company making these filters (A/G Technology) is now producing a 2.75 mm ID filter and this would be recommended for future studies. A larger diameter means an increased cost per unit area, however, so the benefits need to be carefully weighed.

6.2 Cell Recycle Simulation & Costing

The biokinetic model described in the previous section was used to simulate a cell recycle, ethanol fermentation. For a straight fermentor-filter system as was used here, the effect of internal or external bleed is negligible as a large recycle ratio has to be used to obtain reasonable cross-flows over the filter. However, if a centrifuge or other filter arrangement is used, external recycle is advantageous as it allows the bleed ratio to become independent of the fermentor cell concentration (Warren et al., 1989). Further details on some of the preliminary simulation work can be found in Appendix A.

The values found for the parameters in the fermentor and filter equations are given in Table 8, with the values for the cost function equations being given in Table 9.

Table 8 Values of fermentor and filter variables used in the simulation and optimization.

FERMENTOR VARIABLE	μ_m (h^{-1})	K_S (g/L)	P_{m1} (g/L)	a	$(Y_{X/S})_0$ (g/g)	P_{m2} (g/L)	$(Y_{X/P})_0$ (g/g)	P_{m3} (g/g)
VALUE USED	0.43	0.48	103.7	0.65	0.1	165	0.22	165
FILTER VARIABLE	K_{M1}	K_{M2}	K_{M3}	K_{M4}	K_{M5}			
VALUE USED	0.76	-0.1	0.45	1.2	0.0046			

Table 9 Values of the parameters used in the Cost function Equations.

PARAMETER	C_{S1}	C_{F1}	C_{M1}	C_{M2}	C_{M3}	C_{D0}	C_{D1}	C_{D2}	C_{D3}	C_{D4}	C_{D5}
VALUE	0.18	0.68	0.05	0.056	0.001	173.1	-88.1	40.9	1.0	5.0	17.8

Figure 6 demonstrates the effect of varying inlet fermentor substrate and fermentor cell concentrations on the total net operating costs for the variable cell and constant product yield model. From this figure it can be seen that as the cell concentration increases, the cost minima shift to higher substrate concentrations. The minimum at a given cell concentration can also be seen to be fairly flat, an important point for operating conditions as it means that small deviations from the 'optimum' conditions are unlikely to cause major cost increases. Figure 7 shows a breakdown of the costs for varying inlet fermentor substrate concentrations. It is clear that the distillation/drying costs are reduced at higher inlet substrate concentrations due to the higher concentrations of ethanol produced in the fermentor. The membrane cost is also reduced because a lower filtrate flowrate is required per unit of ethanol produced. The factor which causes the cost to increase is the larger fermentor size required to contain the lower dilution rate created by the reduced biomass growth rate.

FIGURE 6. EFFECTS OF FEED SUBSTRATE AND FERMENTOR CELL CONCENTRATIONS ON NET COSTS
(Y_p is constant, Y_x is variable, S = 0.5 g/L.)

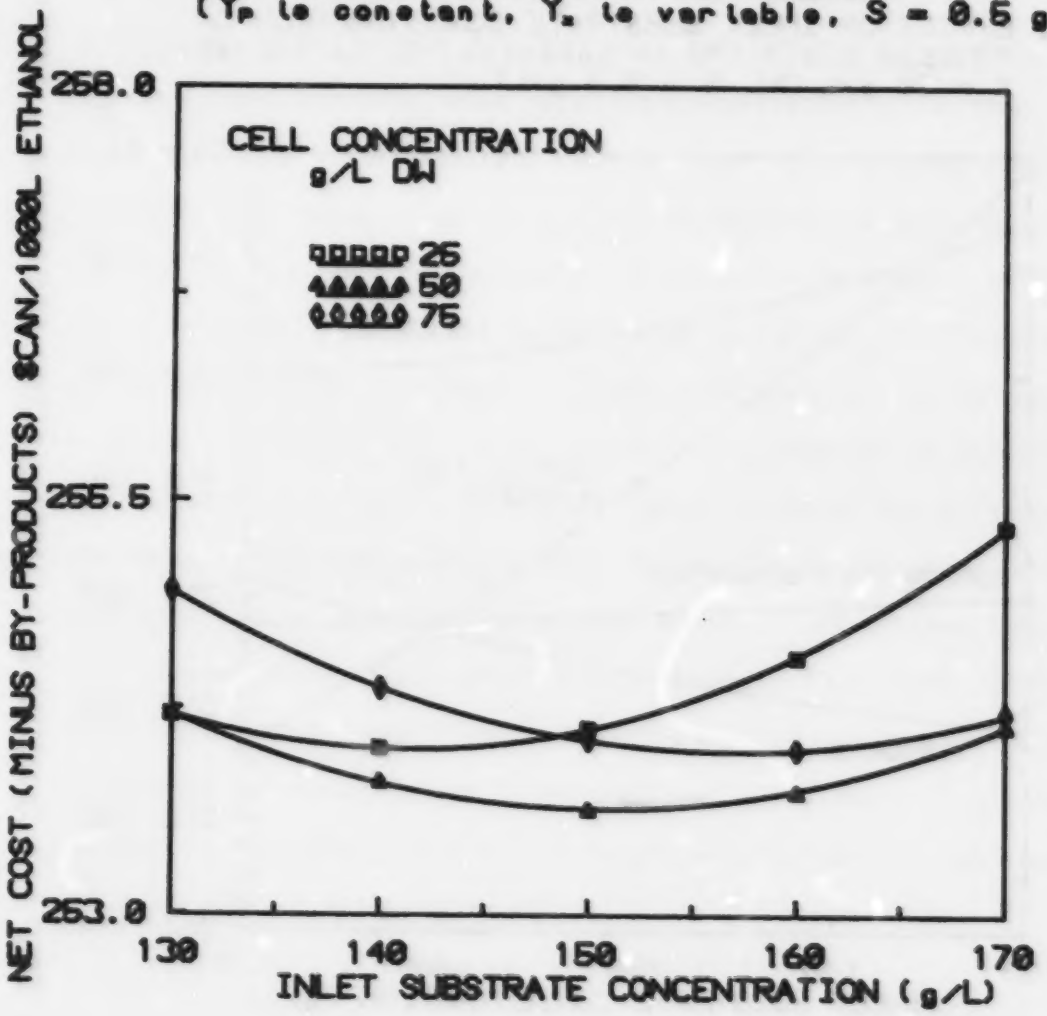
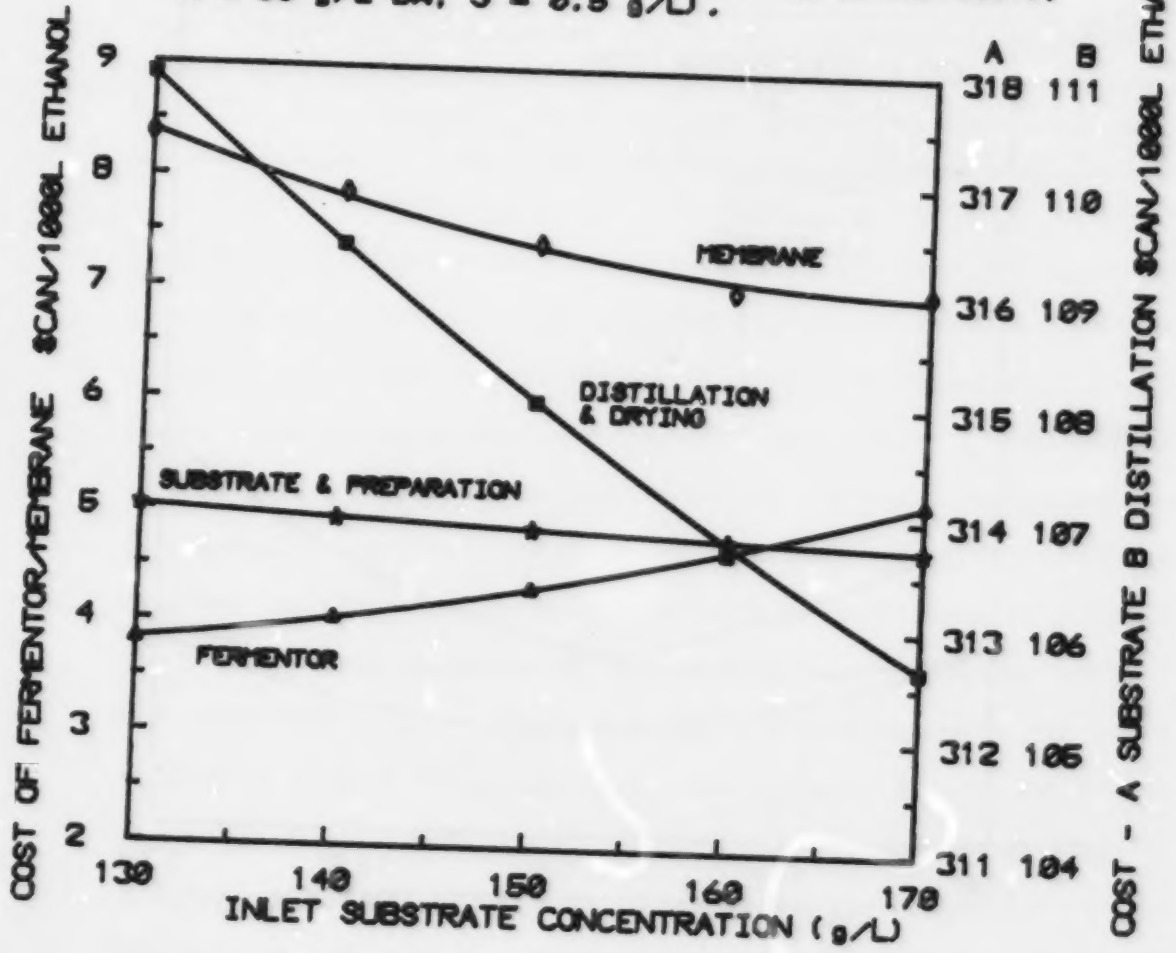


FIGURE 7. EFFECT OF INLET SUBSTRATE CONCENTRATION ON PROCESS COSTS (Y_P is constant, Y_X is variable, X = 50 g/L DW, S = 0.5 g/L).



6.3 Cell Recycle Economic Optimization

The optimization routine was then used to calculate the best conditions for a variety of biokinetic models. The results from this work are given in Table 10, from which it can be seen that significant differences occur between the different models. With a variable cell yield, a lower fermentor inlet substrate concentration is favoured. This lower fermentor inlet substrate concentration in turn favours a lower fermentor cell concentration because: i) the same ethanol productivity can be obtained at a lower cell concentration due to lower product inhibition and ii) a lower fermentor cell concentration will give higher fluxes which will compensate for the higher filtrate flowrate required through the membrane due to a lower outlet product concentration. A significant difference in price occurs as the yield model is changed due to changes in the value of cell and product yields. It can also be seen from Table 10 that if the flux can be doubled through membrane, the optimum fermentor cell concentration also approximately doubles. The inlet fermentor substrate concentrations found from all the models presented here are all lower than the values given by Maiorella et al. (1983). They used no backset, so that sterilization, cooking and evaporation costs decreased as substrate concentration increased.

Table 10 Values of Fermentor Conditions from the Optimization Routine.

MODEL	INLET SUBSTRATE CONCENTRATION (g/L)	FERMENTOR SUBSTRATE CONCENTRATION (g/L)	FERMENTOR CELL CONCENTRATION (g/L)	PROJECTED NET COST ($/1000L ETHANOL)
VARIABLE $Y_{X/S}$, CONSTANT $Y_{P/S}$ (0.46)	150.0	0.67	39.2	253.5
CONSTANT $Y_{X/S}$ (0.08) AND $Y_{P/S}$ (0.46)	182.3	0.84	89.0	238.5
VARIABLE $Y_{X/S}$, CONSTANT $Y_{P/S}$ (0.46) WITH TWICE THE MEMBRANE FLUX	146.9	0.56	61.4	249.8

The first part of Table 11 shows the effect of altering fermentor parameters on the optimum net operating cost for the varying cell and product yield model. The greatest sensitivity is to the product yield, followed by the cell yield, as the most significant cost in producing ethanol is the grain, while an increase in cell yield allows a higher by-product credit. The other parameters affect only the cost of the fermentor, and from Figure 7, it can be seen that changes in the substrate

Table 11 Sensitivity of a) the optimized net operating cost to changes in the fermentor parameters and b) the maximized profit to changes in raw material, energy and product costs.

a)

FERMENTOR PARAMETER	μ_m	K_s	p_{m1}	$(Y_{X/S})_0$	$(Y_{P/S})_0$
% CHANGE IN OPERATING COST PER % CHANGE IN PARAMETER	-0.012	0.004	-0.073	-0.18	-1.0

b)

RAW MATERIAL / ENERGY / PRODUCT	WHEAT	NATURAL GAS	DRIED GRAINS	DRIED YEAST
% CHANGE IN PRE-TAX PROFIT PER % CHANGE IN COST	4.2	0.5	2.1	0.85

cost are much more significant.

Using the prices given in Table 6, it was estimated that the recycle fermentation plant studied here would generate an annual pre-tax profit of $CAN 6 x 10^6. The effect of changing the raw material, energy and by-product selling prices on the profitability of the future plant for the variable cell and product model are shown in the second part of Table 11. Not surprisingly, the parameter with the greatest sensitivity is the wheat cost, where a 10 % cost change can dramatically alter the profitability of the plant.

CONCLUSIONS & RECOMMENDATIONS

1) This study has shown that continuous operation of a fermentor on a lab-scale and at high cell concentrations (50 - 120 g/L) are possible in a fermentor and that significant productivity increases can be achieved over a conventional CSTF. Also, unlike some previous researchers (Lee and Chang,1987, Lafforgue et al., 1987), no cell inhibition was found up to a cell concentration of 120 g/L dry weight. This was probably due to the presence of excess nutrients provided by the yeast extract and air. Whether this would be true in a larger scale system, where mass transfer limitations may play a more significant role is unclear and kinetics in an intermediate scale (20 - 50 L) fermentor are obviously required. This research also used a laboratory prepared media of glucose, yeast extract and salts, so that the effect of a wheat hydrolyzate on kinetics should also be studied. A study on the effects of decreased aeration and free amino nitrogen levels on ethanol productivity would also be interesting.

2) The model for growth rate taken from Warren et al. (1990) was found to fit the experimental data well. As has been previously found by a variety of researchers, including Warren et al. (1989), the cell yield decreases with product concentration. The simple models used here did not match all the data perfectly, which is not suprising due to the complexity of the cell's physiology. Nevertheless, accuracies within ±10% were always achieved. Further data is required at different ethanol and glucose concentrations to improve this model.

3) Although long term operation of the filters was achieved, problems were encountered with plugging and a decline in flux (after 24 hours). The fluxes obtained in these filters is also sub-optimal for

large scale operation, as can be seen by the savings obtained when the flux is doubled. A/G Technology now produce filters with larger diameter tubes. This should reduce avoid plugging by allowing flows at higher Reynolds numbers thereby increasing the fluxes. The disadvantage of a larger area, of course, is a higher cost per unit area. Work is now underway at the University of Saskatchewan to study the effect of diameter on filter operation for yeast microfiltration.

4) When the system was operated at 120 g/LDW, considerable difficulties were faced due to an increase in the amount of foaming. Although the economic analysis suggest that the likely optimized fermentor cell concentration would be in the 30 -70 g/L range, where small amounts of antifoam were sufficient to combat this problem, a new design of fermentor may be needed for high cell concentration operation.

5) Commercial cell systems presently use centrifugation to separate cells for recycle. This work has shown that technically membrane filters are an alternative to centrifuges. A comparison of the economics of the two separators would be useful. Other factors, such as effects on cell viability and the level of contaminations should also be studied.

REFERENCES

A/G Technology Corp., Needham, MA., U.S.A. 1991

Aiba, S., Shoda, M., and Nagatani, M., *Biotechnol. Bioeng.*, 10, 845, 1968.

Allen, A. S., in *8th Energy Biomass Wastes*, Klass, D. L., Ed., (Inst. Gas Technol.:Chicago), 1223, 1984.

Allen, A. S., in *6th Energy Biomass Wastes*, Klass, D. L., Ed., (Inst. Gas Technol.,Chicago) 857, 1982.

Allen, A. S., in *Alternat. Energy Sources IV*, Veziroglu, T. J., Ed., (Ann Arbor Sci.,New York) 3, 421, 1981.

Anonymous, *Chemical Marketing Reporter*, Jan. 14, 1991a.

Anonymous, *Feedstocks*, 63, Jan. 7, 1991b.

Bazua, C.D. and Wilke, C.R.,. Biotechnol. Bioeng. Symp., 7, 105, 1977.

Boks, P. A., and Eybergen, G. C., *Biotechnol. Lett.*, 3, 577, 1981.

Charley, R. C., Fein, J. E., Lavers, B. H., Lawford, H. G., and Lawford, G. R., *Biotechnol. Lett*, 5, 169, 1983.

Cheryan, M. and Mehaia, M., *Biotechnol. Lett.*, 5, 519, 1983.

Cheryan, M., and Mehaia, M., *Proc. Biochem.* 204, 1984.

Cheryan, M., and Mehaia, M. A., *Membrane Separations in Biotechnology*, Mcgregor, W. C., Ed., (Marcel Dekker Inc., New York) 255, 1986.

Cook, R., Wallner, M., and Garlick, L., in *Bioenergy '84*, Egnevs, H., Ellegard, A., Eds., (Elsevier, London) 3, 202, 1984.

Coppella, S. J., and Dhurjati, P., *Chem Eng J.*, 41, B27, 1989

Cysewski, G. R. and Wilke, C. R., *Biotechnol. Bioeng.* 19, 1125, 1977.

Damiano, D., Shin, C., Ju, N., Wang, S. S., *Appl. Microbiol. Biotechnol., 21, 69, 1985.*

Ericsson, E. O., Alcohol from Sulfite Waste Liquor, Chem. Eng. Prog., 43, 165, 1947.

Faust, U., and Knecht, R., in *Proc. 3rd. Internat. Sypos. Fuels Technol.*, Pap. II-40, 1979.

Ghose, T.K. and Tyagi, R.D., *Biotechnol. Bioeng.*, 21, 1401, 1979.

Godia, F., Casas, C. and Sola, C., *Process Biochem.*, 22, 43, 1987.

Grout, H. J., and English, M., in *Energy from Biomass*, Palz, W., Chartier, P., Hall, D., Eds., (Appl. Sci. Pub., London) 360, 1981.

Guidoboni, G. E., *Enzyme Microb. Technol.*, 6, 194, 1984.

Hunt, V. D., *The Gasohol Handbook*, (Ind. Press Inc., New York) 1981.

Jarzebski, A. B., Malinowski, J. J. and Goma, G., *Biotechnol. Bioeng*, 34, 1225, 1989.

Kalk, J. P., and Langlykke, A. F., in *Manual of Industrial Microbiology and Biotechnology*, Demain, A. L., and Solomon, N. A., Eds., (Am. Soc. Micro., Washington) 363, 1986.

Kane, S. M. and Reilly, J. M., Economics of Ethanol Production in the United States. (U. S. Dept. Agricul., Washington) 1989.

Karaki, I. K., Masakazu, A. K., and Ishikawa, F., *Hakko Kyokaishi*. 30, 95, 1972.

Katzen, R., and Assoc., *Grain Motor Fuel Alcohol Technical and Economic Assessment Study*, (U.S. Dept. of Energy, Washington) 1978.

Kuriyama, H., Seiko, Y., Murakami, T., Kobayashi, H. and Sonoda, Y., *J. Ferment. Technol.* , 63, 159, 1985.

Lafforgue, C., Malinowski, J. and Goma, G., *Biotechnol. Lett.*, 9, 347, 1987.

Laluce, C., *CRC Rev. Biotechnol.*, 11, 149, 1991.

Lee, C. W. and Chang, H. N., *Biotechnol. Bioeng.*, 29, 1105, 1987.

Leulliette, L. M., Boudarel, M. J., and Ramirez, A., in *Bioenergy '84*, Egnevs, H., Ellegard, A., Eds., (Elsevier, London) 3, 229, 1984.

Limtong, S., Nakata, M., Funahashi, H., Yoshida, T., Seki, T., Kumnuanta, J., and Taguchi, H., *J. Ferment. Technol.* 61, 55, 1984.

Loser, R. F., *Dev. Ind. Micro.*, 22, 125, 1980.

Luong, J.H.T., *Biotechnol. Bioeng.*, 27, 280, 1985.

Maiorella, B. L., Blanch, H. W., and Wilke, C. R., *Biotechnol. Bioeng.*, 26, 1003, 1983.

Monbouquette, H.G., *Biotechnol. Bioeng.*, **29**, 1075, 1987.

Mota, M., Lafforgue, C., Strehaiano, P., Goma, G., *Bioprocess Eng.*, **2**, 65, 1987.

New Brunswick Scientific Bioflo Model C30 Operating Manual, (New Brunswick Scientific Co. Inc., Edison, NJ) 1989.

Nishizawa, Y., Mitani, Y., Tamai, M., and Nagai, S., *J. Ferment. Technol.*, 6, 599–605, 1983.

Pollard, J. F., *Ethanol Fermentation with Cell Recycle: Mathematical Model and Computer Simulation*. M.Sc. Thesis, Cleveland State Univ., August 1981.

Riggs, J. B., *An Introduction to Numerical Methods for Chemical Engineers*, (Texas Tech. Univ. Press, Lubbock, Texas) **68**, 1988.

Rosen K., *Proc. Biochem.*, 13(3), 25, 1978.

Samejima, H., Nagashima M., Azuma, M., Noguchi, S. and Inuzuka, K., *Ann. New York Acad. Sci.* 434, 394, 1984.

Tutunjian, R.S., *Develop. Ind. Micriobiol.*, **25**, 415–435, 1983.

Ulrich, G. D., *A Guide to Chemical Engineering Process Design and Economics*, (John Wiley & Sons, New York) 1984.

van Gassel, T. J., Ripperger, S., *Desalination*, **53**, 373, 1985.

Warren, R. K., Hill, G. A., and Macdonald, D. G., *Biotechnol. Prog.* 6, 319, 1990.

Warren, R. K., Macdonald, D. G., and Hill, G. A., *Proc. Biochem.*, (In Press) 1991.

Wyman, C. E., and Hinman, N. D., *Appl. Biochem. Biotechnol.*, **24/25**, 735, 1991.

Yarovenko, V. L., *Adv. Biochem. Eng.*, 9, 1, 1978.

Yellow Springs Instrument Model 23A & 23AM Glucose Analyzer Instruction Manual. (Yellow Springs Instrument Co., Yellow Springs, OH) 1989.

Zacchi, G., and Axelsson, A., *Biotechnol. Bioeng.*, **34**, 223, 1989.

APPENDIX A:

PRELIMINARY MODELLING STUDY ON THE EFFECTS OF CELL RECYCLE FOR ETHANOL FERMENTATION

Biotechnol. Prog. 1988, 4, 319–325

Improved Bioreaction Kinetics for the Simulation of Continuous Ethanol Fermentation by *Saccharomyces cerevisiae*

R. K. Warren,* G. A. Hill, and D. G. Macdonald

Chemical Engineering Department, University of Saskatchewan, Saskatoon, Canada S7N 0W0

A kinetic model for the production of ethanol by *Saccharomyces cerevisiae* has been developed from semiempirical analysis. The values for the parameters in this model were then determined by nonlinear multiple regression using the data of Bazua and Wilke (1977). The final equations were $\mu = 0.427s(1 - (p/101.6)^{1.95})/(0.245 + s)$, $Y_{X/P} = 0.291$, and $Y_{X/S} = 0.152(1 - p/302.3)$. This model was then used to simulate a continuous stirred tank fermentor (CSTF) and compared to other models using the same experimental data but different kinetics. The equations required to use these kinetics in a CSTF with recycle were then developed. From this simulation, it was found that, for a CSTF with recycle, the best configuration to operate is an external recycle, with a low bleed and recycle ratio.

Introduction

Microbial biochemistry is a very complex phenomenon based on hundreds of enzyme-controlled reactions, which are in turn influenced by both the previous history of the cell and its surroundings. A structured model that includes all of the necessary equations would therefore contain a very large number of variables and an even larger number of kinetic parameters. Practical fermentor simulations, on the other hand, require a relatively simple model. A careful balance is needed so that the overall kinetics have the minimum number of variables and parameters while still giving a good prediction of the fermentation path. By using the data of Bazua and Wilke (1977), this paper examines the biokinetics of continuous ethanol fermentation and calculates the values of the parameters necessary for the accurate simulation of a fermentor. These are subsequently used in a computer program to predict how values of biomass and ethanol concentrations vary with other variables in a continuous stirred tank fermentor (CSTF) both with and without cell recycle.

Background

Before any process can be efficiently optimized, the kinetics of the system have to be quantified. Work on the kinetics of ethanol fermentation by *Saccharomyces cerevisiae* in continuous systems dates back two decades. It has been found that the fermentation conforms to Monod kinetics, along with noncompetitive ethanol inhibition. Values of μ_m between 0.2 and 0.64 h^{-1} and K_s between 0.22 and 3.3 g/L have been reported (Holzberg et al., 1967; Aiba et al., 1968; Bazua and Wilke, 1977; Ghose and Tyagi, 1979; Hoppe and Hansford, 1982, 1984). Corresponding values for ν_m and K'_s were found to lie between 1.4 and 3.2 h^{-1} and 0.329 and 0.666 g/L, respectively (Aiba et al., 1968; Bazua and Wilke, 1977; Ghose and Tyagi, 1979). A comparison of some of the previous ethanol inhibition models is shown in Figure 1. The variations in these models are partly due to the fact that different yeast strains and different media have been used. However, it can be seen that a variety of models can be used to fit the same data; for example, Bazua and Wilke (1977) and Luong (1985) used very different inhibition equations to model

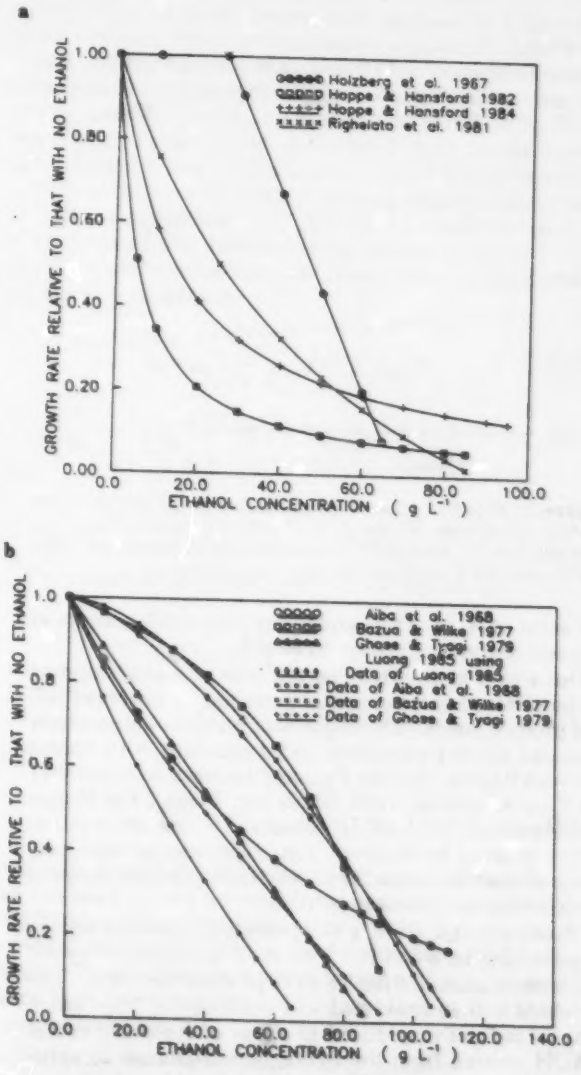


Figure 1. Effect of ethanol concentration on growth rate from previous models: (a) with endogenous ethanol and (b) with exogenous ethanol.

* Author to whom correspondence should be addressed.

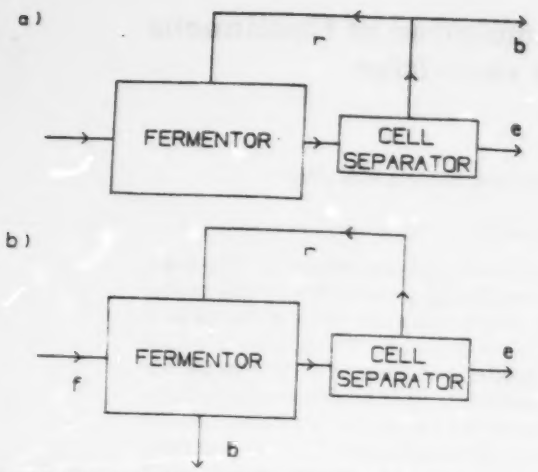


Figure 2. Types of cell recycle fermentors: (a) external recycle and (b) internal recycle.

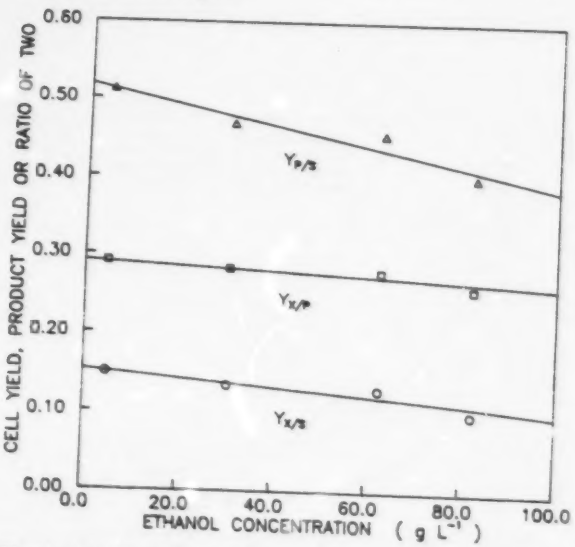


Figure 3. Effect of ethanol concentration on (i) cell yield, (ii) product yield, and (iii) the ratio of cell to product yield. The symbols show the averaged experimental data of Bazua and Wilke (1977), and the lines show the new correlation.

the same data, but these equations gave similar curves at ethanol concentrations up to 90 g/L.

One area of kinetics that has not been extensively studied is the effect of environmental variables on observed cell and product yields. The majority of previous workers have assumed these parameters to be constant with values between 0.09 and 0.1 for $Y_{X/S}$ and between 0.35 and 0.47 for $Y_{P/S}$ (Aiba et al., 1968; Ghose and Tyagi, 1979; Hoppe and Hansford, 1982, 1984). Observed yields are useful as they can easily be obtained from experimental data and, along with an equation for growth rate, can fully describe the kinetics in a fermentor.

Yeasts convert glucose to pyruvate to produce adenosine triphosphate (ATP). This ATP is then used by the cell both to maintain itself and to produce new cells. The pyruvate is then converted to acetaldehyde, from which ethanol is finally produced as a way of removing excess NADH created from the conversion of glucose to pyruvate. The amount of ATP produced should, assuming no respiration occurs, be directly related to the amount of ethanol produced. An energy balance can then be written that

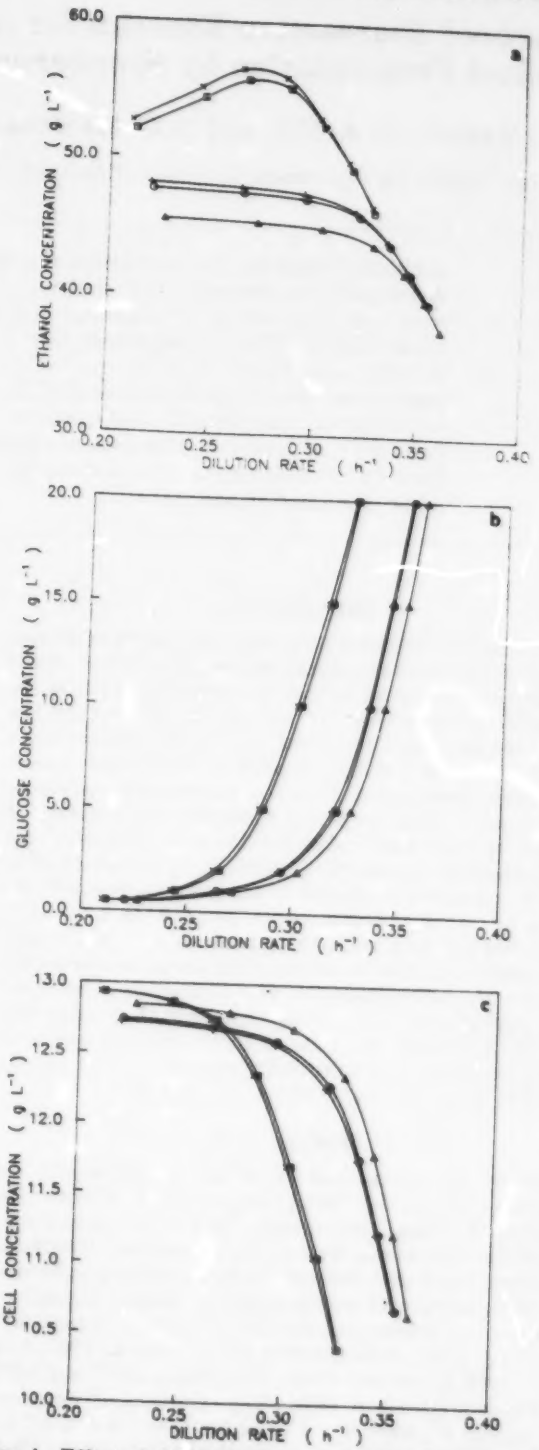


Figure 4. Effect of dilution rate on (a) fermentor ethanol concentration, (b) fermentor glucose concentration, and (c) fermentor cell concentration for a variety of CSTF models at a feed glucose concentration of 100 g/L. ○, new model; +, new model with a variable $Y_{X/P}$; △, new model without taking into account abiotic volumes; □, model using the kinetic model of Bazua and Wilke (1977) for μ and ν; ×, model using the kinetic model of Luong (1985) for μ_m and ν_m.

will relate the yields. If the yields are based on a carbon equivalent substrate basis (Andrews, 1989), this gives

$$N\hat{Y}_{P/S} = \hat{Y}_{X/S}/\hat{Y}_{ATP} + \hat{m} \qquad (1)$$

If the medium has an organic source close to the composition of the cells that can be metabolized by the yeast and if the fermentation produces a single product, then (Andrews, 1989)

$$Y_{P/S}/Y_{P/S_m} = 1 \quad Y_{P/S} = {}^2/_3 \tag{2}$$

Three problems, however, occur when applying these equations. The first of these is that m and Y_{ATP} may not be constant. The second problem is that byproduct formation has not been taken into account in the analysis. Finally, if oxygen is present, some aerobic growth may occur. Because of these problems, a theoretical model derived in a totally quantitative fashion would be complicated and involve many uncertain parameters. Instead, this paper presents a more qualitative approach in which the expected overall effect of environmental variables on the kinetic parameters is first found, from which an unstructured model for $Y_{X/S}$ and $Y_{X/P}$ is derived, on the basis of those external variables. This model is then applied to the experimental data of Bazua and Wilke (1977) to obtain quantitative values for the parameters in these equations.

At low ethanol concentrations, the data of Bazua and Wilke (1977) showed ethanol yields around Y_{P/S_m}, suggesting that almost all the glucose added is used to form ethanol. Bazua and Wilke (1977) used significant amounts of yeast extract in their medium (1.5 g/L compared to 10 g/L glucose). Yeast extract is formed by autolysing yeast and contains around 14% carbohydrate (Rohne, 1973) and a plentiful supply of various amino acids [about 5.5% free amino nitrogen (Rohne, 1973)] that can easily be used to build cells. The cell concentration in the fermentor was always much lower than that of the added yeast extract. It is possible, therefore, that the majority of the cellular material could come from the yeast extract. For both these reasons it seems reasonable to use eq 2 as a starting point for $Y_{P/S}$. However, the presence of byproducts has not been taken into account. It has been shown (Oura, 1977) that if nitrogen is not limiting, the major byproduct will always be glycerol. Other byproducts are usually produced in significantly smaller amounts that it is reasonable to ignore them. Glycerol is thought to be produced either as a means of removing excess NADH (Oura, 1974) or as a relatively nontoxic solute by which the cell's osmotic pressure can be increased at low water activities (a_w) (Kenyon et al., 1986). An increase in ethanol concentration will cause an increase in acetaldehyde production, leading to higher levels of NADH (Jones, 1989) and will also cause a_w to decrease. For either explanation the glycerol concentration will increase, so decreasing $Y_{P/S}$.

$Y_{X/P}$ will also decrease with increased ethanol concentration for two reasons: (i) m will increase because the cell consumes more energy counteracting the lower a_w and the inhibitory effects of ethanol and acetaldehyde and (ii) the conversion of 0.5 mol of glucose to 1 mol of glycerol consumes 1 mol of ATP, so less energy from ethanol production is available for cell production. No data for the effect of environmental variables on Y_{ATP} are available, but assuming that the respiratory coefficient does not change, it is probable that Y_{ATP} is relatively constant. Similarly, the direct effects of cell concentration and substrate concentration on m are likely to be minimal for typical conditions.

As the growth rate decreases, m will increase and so $Y_{X/P}$ should decrease. However, generally this effect is only significant at low growth rates, and in experimental results it is usually masked by an increase in the respiratory coefficient, so that $Y_{X/P}$ actually increases. For the purpose of this model the effects of growth rate have been ignored, although in further models we plan to include them. Because of this, one proviso on the model is that it should not be used at low growth rates. As most fermentations are normally run at growth rates above 0.1 h^{-1} to give a reasonable productivity, this should not cause a serious user constraint.

From the above arguments, $Y_{P/S}$, $Y_{X/P}$, and so $Y_{X/S}$ should decrease with increasing ethanol concentration. Previous results have shown a decrease in $Y_{X/S}$ with increasing ethanol concentration, supporting this theory (Suzuki et al., 1972; Duvnjak et al., 1980; Orlava et al., 1980).

Model Equations

For a CSTF model, three kinetic equations are required to determine growth rate, μ, specific substrate uptake, q, and specific ethanol production rate, ν. It was decided to use one equation for μ and then link q and ν to μ by using $Y_{X/S}$ and $Y_{X/P}$. The Monod equation is used along with the product inhibition term given by Luong (1985) to model μ. For the reasons outlined above, both $Y_{X/S}$ and $Y_{X/P}$ were expected to decrease with increasing ethanol concentration. From our regression analysis studies, we found that the increase in accuracy from using nonlinear equations did not compensate for the significant increase in the complexity of the model compared to linear equations. These results also showed that, for the data of Bazua and Wilke (1977), $Y_{X/P}$ was found to vary by only 10% over their whole range of ethanol concentrations, so that use of a constant $Y_{X/P}$ gave only a small deviation. This allowed the fermentor equations to be solved explicitly, thus saving computer time. The equations used for the new model are

$$\mu = \mu_m \frac{s}{(s + K_s)}[1 - (p/p_{m1})^a] \tag{3}$$

$$Y_{X/S} = (Y_{X/S})_0(1 - p/p_{m2}) = \mu/q \tag{4}$$

and

$$Y_{X/P} = (Y_{X/P})_0 = \mu/\nu \tag{5}$$

The values of the parameters in the equations

$$\nu = \nu_m \frac{s}{(K'_s + s)}(1 - p/p'_m) \tag{6}$$

$$Y_{X/P} = (Y_{X/P})_0(1 - p/p_{m3}) \tag{7}$$

and

$$Y_{P/S} = (Y_{P/S})_0(1 - p/p_{m4}) \tag{8}$$

were calculated for comparative purposes, although they are not used in the final fermentor model. A commercial nonlinear multiple regression technique (UNSLF, marketed by IMSL, Houston, TX) was used for parameter identification. Values of standard errors for the model parameters were then calculated by using Jacobians at the least-squares locations.

Yields are based on abiotic phase volumes:

$$Y_{X/S} = \frac{x}{s_p - s(1 - x/\rho)} \tag{9}$$

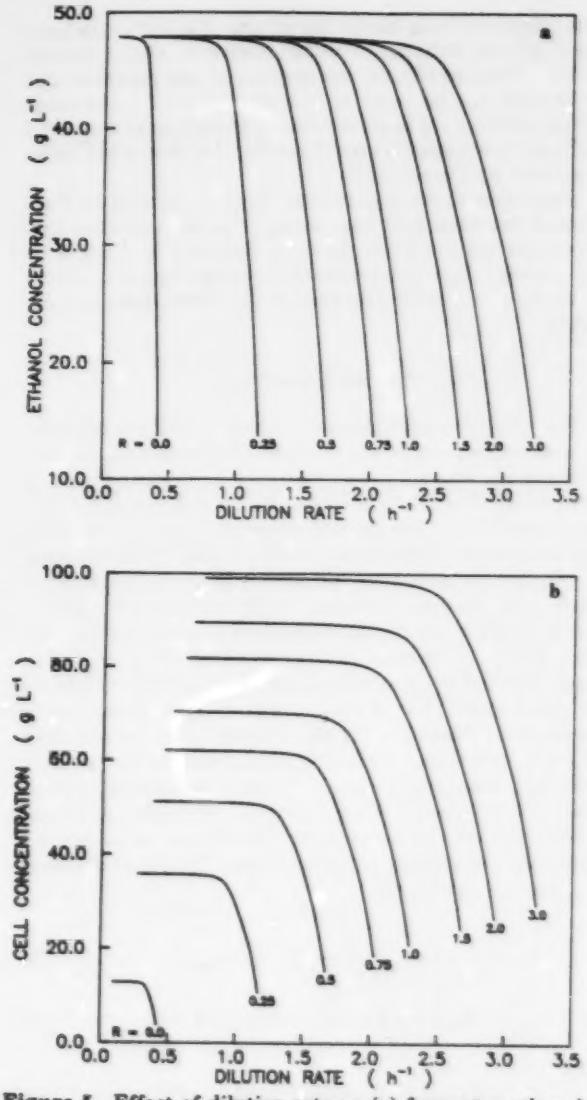


Figure 5. Effect of dilution rate on (a) fermentor ethanol concentration and (b) fermentor cell concentration at varying recycle ratios for external recycle with a feed glucose concentration of 100 g/L and a bleed ratio of 0.1.

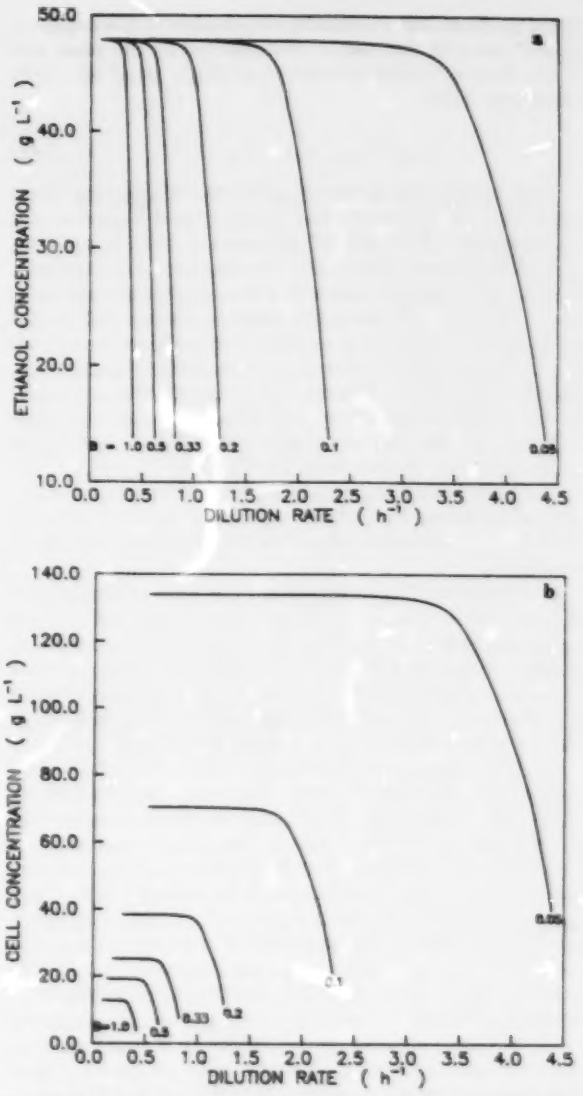


Figure 6. Effect of dilution rate on (a) fermentor ethanol concentration and (b) fermentor cell concentration at varying bleed ratios for external recycle with a feed glucose concentration of 100 g/L and a recycle ratio of 1.0.

$$Y_{X/P} = \frac{x}{p(1 - x/\rho) - p_F} \tag{10}$$

$$Y_{P/S} = \frac{p(1 - x/\rho) - p_F}{s_F - s(1 - x/\rho)} \tag{11}$$

Fermentor Simulation

The optimized biokinetic model can now be used to model a continuous ethanol fermentation. The equations derived below are for a CSTF with external cell recycle (Figure 2a), but they can easily be modified for a CSTF with internal cell recycle (Figure 2b) or for a straight CSTF. The simulation is designed to be used as part of a larger system that has an overall recycle from a product separation device, so that some product may be found in the feed. The model takes into account the effect of biomass volume fractions by using abiotic and total volumes (Monbouquette, 1987). The known parameters are feed substrate concentration, s_F, feed product concentration, p_F, and fermentor abiotic phase substrate concentration, s. Values of fermentor abiotic phase product concentration, p, dilution rate, d, and fermentor cell concentration, x, are then calculated. The equations are solved from the following five algebraic mass balances.

(i) Total flow rate around whole system:

$$B + E = 1 \tag{12}$$

(ii) Biomass balance around whole system:

$$\frac{d(xV_r)}{dt} = [1 - (p/p_{m1})^a]\mu_m \frac{sxV_r}{(K_s + s)} - bx_b \tag{13}$$

(iii) Biomass balance around cell concentrator:

$$x_b/x = (R + 1)/(R + B) = \kappa \tag{14}$$

Substituting eq 14 into eq 13 and making it dimensionless gives

$$\frac{dX}{dT} = (1 - P^a)\frac{S}{(S + 1)}X - DBX\kappa \tag{15}$$

(iv) Substrate balance around whole system:

$$\frac{d[(1 - x/\rho)sV_r]}{dt} = fs_F - b(1 - x_b/\rho)s - es - \frac{\mu_m[1 - (p/p_{m1})^a]sxV_r}{(Y_{X/S})_0(1 - p/p_{m2})(K_s + s)} \quad (16)$$

Substituting eqs 12 and 14 into eq 16 and making it dimensionless gives

$$\frac{dS}{d\bar{T}} = \frac{(S_F - S)}{(1 - X)}D - \frac{(1 - P^a)SX}{(1 - X)(S + 1)}\left[\frac{\phi_0}{(1 - PK_2)} - S\right] \quad (17)$$

(v) Product balance around whole system:

$$\frac{d[1 - (x/\rho)pV_r]}{dt} = fp_F - b(1 - x_b/\rho)p - ep + \frac{\mu_m[1 - (p/p_{m1})^a]sxV_r}{Y_{X/P}(K_s + s)} \quad (18)$$

which transforms to the dimensionless equation

$$\frac{dP}{d\bar{T}} = \frac{(P_F - P)}{(1 - X)}D + \frac{(1 - P^a)SX}{(1 - X)(S + 1)}(\pi + P) \quad (19)$$

Assuming steady state, these five equations can be combined to give a quadratic for P. Letting $S_R = S/S_F$ and $\phi_R = \phi_0/S_F$ gives

$$P = {}^1/_2\left\{\pi(S_R - 1) + P_FS_R + \frac{1 - \phi_R}{K_2} + \left[(\pi(S_R - 1) + P_FS_R)^2 + \frac{(1 - \phi_R)^2}{K_2} - \frac{2}{K_2}((\pi(S_R - 1) + P_FS_R)(1 + \phi_R) - 2P_F\phi_R)\right]^{1/2}\right\} \quad (20)$$

Then

$$D = \frac{S(1 - P^a)}{(S + 1)B\kappa} \quad (21)$$

and

$$X = \frac{(S_F - S)(1 - K_2P)}{B\kappa[\phi_0 - S(1 - K_2P)]} \quad (22)$$

For a variable $Y_{X/P}$, where $Y_{X/P} = (Y_{X/P})_0(1 - K_3P)$, a similar derivation gives a cubic for P, which can be solved by using the Newton–Raphson method:

$$f(P) = S_FK_2K_3P^3 + [\phi_0K_3 - (K_2 + K_3)S_F - K_2K_3SP_F]P^2 + [(S - S_F)\pi_0K_2 - \phi_0(P_FK_3 + 1) + P_FS \times (K_2 + K_3) + S_F]P + (S_F - S)\pi_0 + P_F(\phi_0 - S) = 0 \quad (23)$$

after which D and X can be obtained from eqs 21 and 22.

One further modification is that suggested by Lee et al. (1983). They found that, at high cell concentrations, cell inhibition of growth rate occurs. This can be modeled by adding a term, $1 - X$, to the expression for μ. The only equation this changes is eq 21, which then becomes

$$D = \frac{S(1 - P^a)(1 - X)}{(S + 1)B\kappa} \quad (24)$$

To use the model as a CSTF with internal cell recycle, the only change needed is to let κ equal 1, while a straight CSTF with no cell recycle can be obtained by letting both κ and B equal 1.

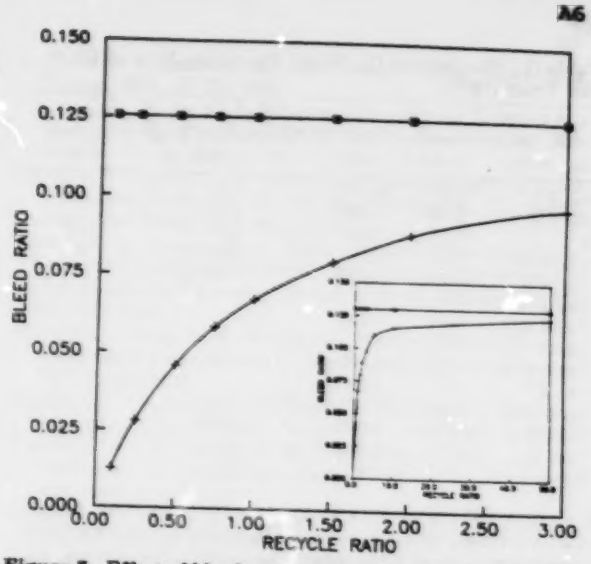


Figure 7. Effect of bleed ratio on recycle ratio at a fermentor cell concentration of 100 g/L and a feed glucose concentration of 100 g/L. +, external recycle; □, internal recycle. Inset shows values for higher recycle ratios.

Table I. Parameters for Model Fits of the Data of Bazua and Wilke (1977)

parameter	new model	std error	model of Bazua and Wilke (1977)	model of Luong (1985)
μ_m, h^{-1}	0.427	0.0004	0.428	
K_s, g/L	0.245	0.0005	0.238	
a	1.95	0.004		1.65
p_{m1}, g/L	101.6	0.06	93.8	107.8
Y_{X/S_0}, g/g	0.152	0.0004		
p_{m2}, g/L	302	4.2		
Y_{X/P_0}, g/g	0.291	0.001		
p_{max}, g/L	1050	86		
Y_{P/S_0}, g/g	0.518	0.001		
p_{ast}, g/L	396	6.8		
π_0, g/(g·h)	2.08	0.05	1.80	
K'_s, g/L	0.34	0.07	0.33	
c	2.16	0.09		1.81
p'_m, g/L	106.0	1.3	93.1	114

Results and Discussion

Kinetic Parameters. The simulation used is based on the data generated by Bazua and Wilke (1977) for *S. cerevisiae* ATCC 4126. However, the model could easily be applied to other fermentations by changing the biokinetic parameters. The data of Bazua and Wilke (1977) were used because they gave tabulated values over the widest range of ethanol concentrations. Only 12 of their 20 reported points could be used because eight of the runs had inlet and fermentor product concentrations so close that reliable values for the product yield could not be determined. Another problem with the use of their data in a CSTF simulation is that the data were found with exogenous rather than endogenous ethanol. From Figure 1, it can be seen that, in general, the growth rate decreases faster as the ethanol concentration increases with endogenous as compared to exogenous ethanol, because of which the model may overpredict the dilution rate.

The parameter values for the data of Bazua and Wilke (1977) from three different models are shown in Table I, along with the standard error for the new model. The small standard errors indicate that the new model fits the experimental data extremely well. The values for a, c, p_{m1}, and p'_m are significantly different from those given by Luong (1985). This is probably because Luong (1985) used

Table II. Parameters for Model Fits of the Data of Ghose and Tyagi (1979)

parameter	new model	std error	model of Ghose and Tyagi (1979)	model of Luong (1985)
μ_m, h^{-1}	0.357	0.0003	0.4	
K_s, g/L	0.38	0.0006	0.476	
a	1.11	0.003	1.0	0.8
p_{m1}, g/L	95.0	0.1	87.0	92.7
$Y_{X/S0}$, g/g	0.11	0.0001	0.09	
p_{m2}, g/L	374	4.1		
$Y_{X/P0}$, g/g	0.34	0.001		
p_{m3}, g/L	660	40		
$Y_{P/S0}$, g/g	0.31	0.00004	0.47	
p_{m4}, g/L	776	2.33		
r_m, g/(g·h)	1.45	0.004	1.4	
K'_s, g/L	0.70	0.004	0.666	
c	1.57	0.01	1.0	1.0
p'_m, g/L	90.9	0.3	114	114.5

a three-point graphical fit based on the maximum values of μ calculated by Bazua and Wilke (1977), while multiple regression allowed 12 points to be used by the new model.

The values for $(Y_{X/S})_0$, $(Y_{X/P})_0$, $(Y_{P/S})_0$, p_{m1}, p_{m2}, and p_{m3} cannot be compared with previous models, but reasonable fits (Figure 3) for the observed yields were found, and from the data it was obvious that they decreased as P increased, supporting our previous hypothesis.

Values for the new parameters were also found from the ethanol fermentation data of Ghose and Tyagi (1979), as shown in Table II along with two previous models. The values found were generally within 10% of those previously reported. As with the data of Bazua and Wilke (1977), our new model should give more accurate results because it uses considerably more points and does not have the inaccuracies due to graphical approximations.

Fermentor Simulations. The fermentor simulation was first used as a CSTF. Figure 4 shows a comparison of the various models in a simulation of a CSTF as dilution rate varies. The curve labeled Bazua used the correlation given by Bazua and Wilke (1977) for μ and ν. The curve labeled Luong used the correlations given by Luong (1985) for ethanol inhibition, taken from the results of Bazua and Wilke (1977), along with the latter's data for μ_m, ν_m, K_s, and K'_s. An average value of 0.13 g/g for $Y_{X/S}$ was also used in both cases. Even though these kinetic models are very different, the curves are very similar. One problem they both have is they predict an initial increase in product concentration with dilution rate, while it should decrease. The results of a simulation using the new kinetics, but first without taking into account the cells' volumes and second using kinetics in which $Y_{X/P}$ is varied as in eq 7, are also shown. From these curves, it can be seen that the change caused by taking into account the cells' volume in an abiotic simulation is significant, while not varying $Y_{X/P}$ produces little error. As mentioned earlier, use of a constant $Y_{X/P}$ allows an explicit solution to be used.

Figures 5 and 6 show the effect of varying variables in a CSTF with external recycle, from which it can be seen that cell concentration and product concentration increase as the recycle ratio increases and the bleed ratio decreases. However, the bleed and recycle ratios can only be increased to a certain point, because at high cell concentrations, problems with pumping in the recycle stream or with mass transfer in the fermentor will occur. A limit on bleed ratio may also occur due to the buildup of toxic products.

The final figure (Figure 7) shows the value of bleed ratio at varying recycle ratios for a fixed feed substrate concentration and a fixed fermentor cell concentration. For an external recycle, as the recycle ratio increases, the bleed ratio also increases, until in the limit it reaches that for an internal recycle. This shows one of the advantages of external recycle, because a lower bleed ratio can be used at the same cell concentration as that for internal recycle. It also shows that the best operating conditions for an external recycle system are likely to be at a low bleed ratio and a low recycle ratio, because here a reasonable cell concentration can be maintained, giving a high productivity with high yield in the fermentor, while also allowing most of the product to leave in the product stream.

The simulation could be improved by (i) taking into account oxygen, if present, along with the effect of changing dilution rate and (ii) taking into account the effect of inhibitors other than ethanol, which may become important at higher cell concentrations. However, further experimental data are required for this.

Conclusions

A model for ethanol fermentation kinetics developed from theoretical analysis has been shown to fit the data of both Bazua and Wilke (1977) and Ghose and Tyagi (1979). These kinetics were then used in a simulation of a CSTF, from which a model using abiotic volumes and using a growth rate and cell yield that vary with ethanol concentration was determined to be the best balance of usability and accuracy.

By using this simulation as a CSTF with recycle, it was found that higher dilution rates could be achieved as the bleed ratio was decreased and, in the case of external recycle, as the recycle ratio was increased. For a recycle system, the bleed ratio should be as low as possible, as this allows the least amount of product to be lost with the cells. For a CSTF with external recycle at a fixed fermentor cell concentration, it was found that the bleed ratio increased with the recycle ratio. As one of the constraints on a CSTF recycle system is the cell concentration in the fermentor, the best place to operate the system is likely to be at a low bleed and low recycle ratio.

Notation

Generally, small letters indicate actual quantities and large letters indicate dimensionless quantities.

a	product inhibition power for cell growth
b	bleed stream flow rate, L/h
B	ratio of bleed stream flow rate to feed stream flow rate (b/f)
c	product inhibition power for ethanol production
D	dimensionless dilution rate $[f/(V_v\mu_m)]$
e	filtrate stream flow rate, L/h
E	ratio of filtrate stream flow rate to feed stream flow rate (e/f)
f	feed stream flow rate, L/h
K_s	Monod constant for μ, g/L
K_2	ratio of product inhibition constant for μ to $Y_{X/S}$ (p_{m1}/p_{m2})
K_3	ratio of product inhibition constant for μ to $Y_{X/P}$ (p_{m1}/p_{m3})
m	moles of ATP used for maintenance per mole of carbon equivalent substrate consumed
N	moles of ATP generated per mole of carbon equivalent substrate converted to ethanol
p	fermentor abiotic phase product concentration, g/L
p_{m1}	maximum abiotic phase product concentration for cell growth, g/L
p_{m2}	product inhibition constant for $Y_{X/S}$, g/L
p_{m3}	product inhibition constant for $Y_{X/P}$, g/L
p_{m4}	product inhibition constant for $Y_{P/S}$, g/L

P	dimensionless fermentor product concentration, p/p_{m1}
q	specific substrate uptake rate, h^{-1}
r	cell recycle stream flow rate, L/h
R	ratio of cell recycle stream flow rate to feed stream flow rate (r/f)
s	fermentor abiotic phase substrate concentration, g/L
S	dimensionless fermentor substrate concentration (s/K_s)
S_R	dimensionless reduced substrate concentration (S/S_F)
t	time, h
T	dimensionless time ($t\mu_m$)
V_r	working volume of fermentor including cells, L
x	fermentor cell dry weight concentration, g/L
x_b	bleed stream cell dry weight concentration, g/L
X	dimensionless cell concentration (x/ρ)
Y_{ATP}	moles of ATP required to form a mole of carbon equivalent cells from the molecules present in the medium, mol/mol
$Y_{P/S}$	product mass yield from the substrate, g/g
$Y_{X/P}$	ratio of cell dry weight yield to product mass yield ($Y_{X/S}/Y_{P/S}$)
$Y_{X/S}$	cell dry weight yield from the substrate, g/g

Superscripts

*	corresponding carbon equivalent value
′	corresponding value for ν rather than μ

Subscripts

m	maximum possible value
F	feed stream
0	value at zero product concentration

Greek Symbols

κ	ratio of cell dry weight concentration in the bleed stream to that in the fermentor (x_b/x)
μ	specific growth rate, h^{-1}
π	dimensionless constant $[\rho/(Y_{X/P}p_{m1})]$
ρ	cell dry weight per unit volume of wet biomass, g/L
ϕ	dimensionless constant $[\rho/(Y_{X/S}K_S)]$
ϕ_R	dimensionless constant (ϕ_0/S_F)
ν	specific ethanol production rate, g/(g·h)

Literature Cited

Aiba, S.; Shoda, M.; Nagatani, M. Kinetics of Product Inhibition in Alcohol Fermentation. *Biotechnol. Bioeng.* **1968**, *10*, 845–864.

Andrews, G. Estimating Cell and Product Yields. *Biotechnol. Bioeng.* **1989**, *33*, 256–265.

Bazua, C. D.; Wilke, C. R. Ethanol Effects on the Kinetics of a Continuous Fermentation with *Saccharomyces cerevisiae*. *Biotechnol. Bioeng. Symp.* **1977**, *7*, 105–118.

Duvnjak, Z.; Kosaric, N. In *Advances in Biotechnology: v2 Fuels, Chemicals, Foods and Wastes*; Moo-Young, M., Ed.; Pergamon: New York, 1980; pp 175–180.

Ghose, T. K.; Tyagi, R. D. Rapid Ethanol Fermentation of Cellulose Hydrolysate II Product and Substrate Inhibition and Optimization of Fermentor Design. *Biotechnol. Bioeng.* **1979**, *21*, 1401–1420.

Holzberg, I.; Finn, R. K.; Steinkraus, K. H. A Kinetic Study of the Alcoholic Fermentation of Grape Juice. *Biotechnol. Bioeng.* **1967**, *9*, 413–427.

Hoppe, G. K.; Hansford, G. S. Ethanol Inhibition of Continuous Anaerobic Yeast Growth. *Biotechnol. Lett.* **1982**, *4*, 39–44.

Hoppe, G. K.; Hansford, G. S. The Effect of Micro-Aerobic Conditions on Continuous Ethanol Production. *Biotechnol. Lett.* **1984**, *6*, 681–686.

Jones, R. P. Biological Principles for the Effects of Ethanol. *Enzyme Microb. Technol.* **1989**, *11*, 130–153.

Kenyon, C. P., Prior, B. A.; van Vuuren, H. J. J. Water Relations of Ethanol Fermentation by *Saccharomyces cerevisiae*: Glycerol Production under Solute Stress. *Enzyme Microb. Technol.* **1986**, *8*, 461–464.

Lee, J. M.; Pollard, J.; Coulman, G. Ethanol Fermentation with Cell Recycling: Computer Simulation. *Biotechnol. Bioeng.* **1983**, *25*, 497–511.

Luong, J. H. T. Kinetics of Ethanol Inhibition in Alcohol Fermentation. *Biotechnol. Bioeng.* **1985**, *27*, 280–285.

Monbouquette, H. G. Models for High Cell Density Bioreactors must Consider Biomass Volume Fraction: Cell Recycle Example. *Biotechnol. Bioeng.* **1987**, *29*, 1075–1080.

Orlova, V. S.; Semikhatara, N. M.; Rylkin, S. S. Effect of the Concentration of Ethanol on Physiological Properties of *Saccharomyces cerevisiae* during Batch Cultivation. *Mikrobiol. Zh. (Kiev)* **1980**, *42*, 718–722.

Oura, E. Effect of Aeration on the Biochemical Composition of Baker's Yeast 1 Factors Affecting the Type of Metabolism. *Biotechnol. Bioeng.* **1974**, *16*, 1197–1212.

Oura, E. Reaction Products of Yeast Fermentations. *Process Biochem.* **1977**, *12* (3), 19–22.

Righelato, R. C.; Rose, D.; Westwood, A. W. Kinetics of Ethanol Production by Yeast in Continuous Culture. *Biotechnol. Lett.* **1981**, *3*, 3–8.

Rohne, P. A. *BBL Manual of Products and Laboratory Procedures: 5th Ed.*; Becton Dickinson Co.: Cockeysville, MA, 1973; p 163.

Suzuki, A.; Nishya, T.; Shigaki, K. Kinetic Studies on Yeast Growth (Part 4), Effects of the Concentration of Ethanol on Physiological Properties of *Saccharomyces cerevisiae* during Batch Cultivation. *J. Soc. Brew., Jpn.* **1972**, *67*, 449–452.

Accepted July 24, 1990.

Registry No. Ethanol, 64-17-5.

APPENDIX B:

PRELIMINARY DESIGN AND COSTING OF A CONTINUOUS ETHANOL PROCESS FOR WESTERN CANADA USING CELL RECYCLE

Plant Design

This design is based on a 100 million L/year 99.2 % ethanol capacity. The site is based near an urban centre in Saskatchewan, using feed wheat grown nearby and with the dried grains and solubles (DGS) and dried yeast by-products being sold to local farmers. A simplified flowsheet of the plant is shown in Figure 1. The process has a number of novel steps including: i) separation of the suspended solids before saccharification, ii) an immobilized enzyme packed column for saccharification, and iii) a cross-flow membrane filter to allow cell recycle in the fermentor. These changes have also allowed for improvements in the rest of the plant, such as allowing sieve plates to be used in the rectifier without regular cleaning and allowing a higher soluble solids concentration to exit from the evaporator. Most of the equipment in the plant was estimated from a previous report by R. Katzen Assoc. (1978) and from Ulrich (1984). The plant generates its own steam from a gas fired boiler and includes waste water treatment and a cooling tower. A brief description of each stage in the process is given in the following paragraphs.

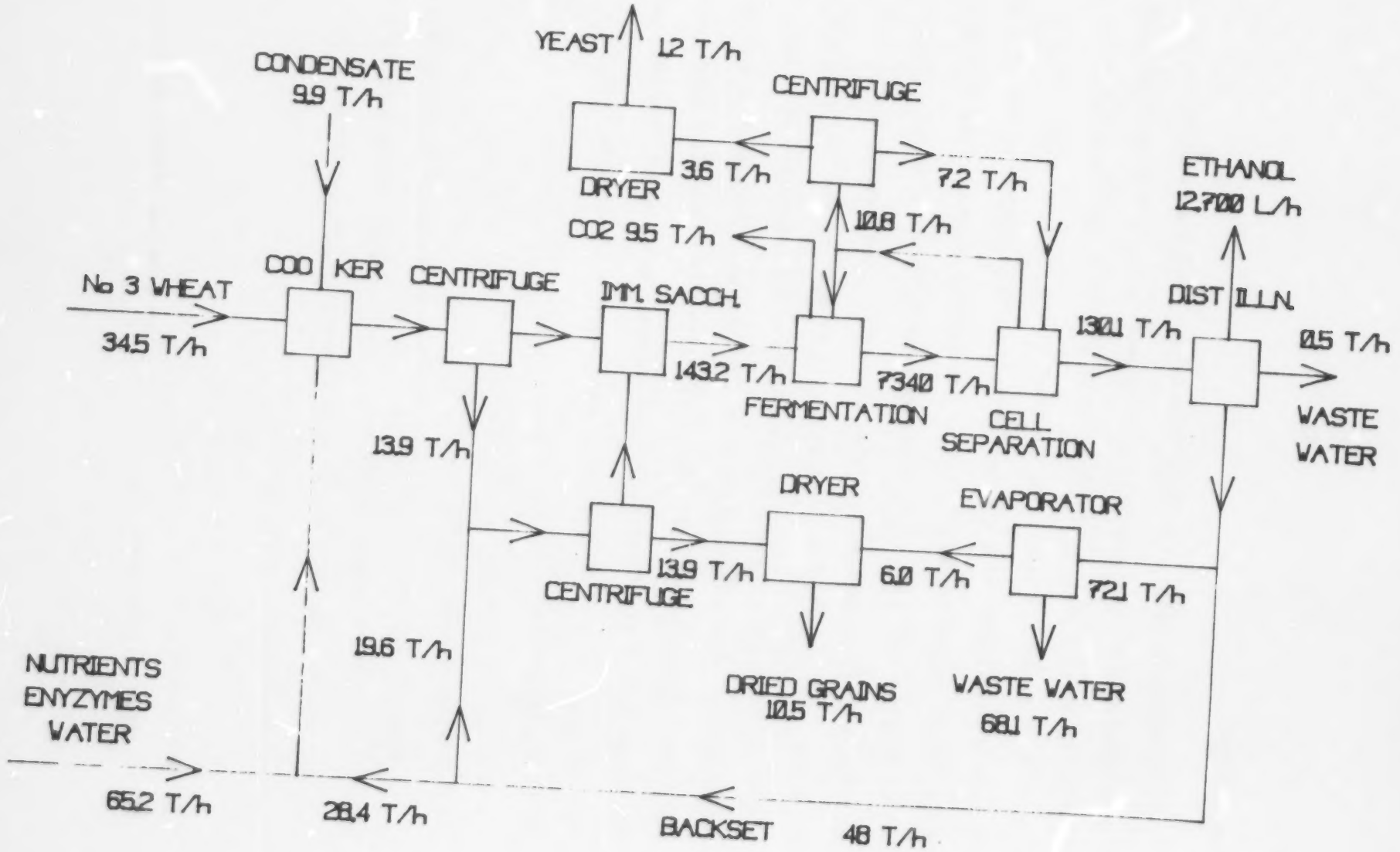
a) Receiving, Storage and Milling.

Wheat is delivered by railroad, weighed and conveyed to storage bins and hence to the surge hopper. A cleaner is then used to remove metal and large 'tramp' objects, the grain is milled in hammer mills, passed through a star valve and conveyed to the cooking section.

b) Mash Cooking.

After being weighed, the wheat is mixed with new process water, backset,

Figure 1 Schematic diagram of the 100 million L/yr. ethanol plant showing the stages in the plant and inter-stage flows.



gelatinizing enzymes and condensate from the pressure flash tanks in the mash mixing tank. From here the mash is transferred to the mash pre-cooker before being further heated in the mash heater to 110 ° C using 2 bar steam. In the cooker 12 bar live steam is used to achieve a temperature of 177 ° C for 1.5 minutes. The mash is then flashed twice and cooled to 40 ° C. The flashed steam is then used to either preheat the mash or the feed to the distillation column.

c) Suspended Solid Separation.

In order to use the saccharification and cell recycle stages in this process, the suspended solids must first be separated. This prevents the suspended solids from plugging the saccharification column and from interfering with the fermentor operation. A decanter centrifuge is used for this separation. When separating out the suspended solids in a mash solution, there is a maximum of 40 - 45 % solids in the concentrate. As such, some of the dextrins would also be lost. As this would significantly reduce the overall wheat to ethanol yield, these dextrins need to be recovered. The best way to do this is to wash the solids. In the system suggested here, this is done by mixing the solid with part of the hot backset and then centrifuging this mixture. The liquid from this centrifuge is then joined with that coming from the other centrifuges and, after cooling, is sent to the saccharification section.

d) Saccharification.

After the starch has been gelatinized, the sugars are mainly in the form of dextrins. These dextrins have to be broken down by saccharifying enzymes to units of sugar molecules of three or less. For the industrial loadings of enzymes commonly used, this would take 24 hours, so for a typical batch fermentation,

simultaneous saccharification and fermentation is used. However, because of the short residence times for a continuous cell recycle fermentor, saccharification needs to be completed before fermentation. Higher enzyme loadings are then required and, because this would be very costly if free enzymes were used, an immobilized enzyme system is used, with loadings of 0.2 g/g support. This allows a high conversion of the dextrins to utilizable sugars with a residence time of less than one hour. Packed columns of immobilized amyloglucosidase have been tested industrially in the starch processing industry, where the enzyme needed to be replaced after 6 months (Daniels, 1986). After saccharification, the solution is passed to the fermentor holding tank.

e) Fermentation.

The mash entering the fermentor contains approximately 15.8 % (wt./vol) utilizable sugars and these are almost totally converted to alcohol (0.1 % residual sugar). The fermentation is continuous and uses cell recycle to maintain a cell concentration of 100 g/L DW in the fermentor. With this concentration, a residence time of less than two hours can be used, so that the fermentor volume is considerably reduced compared to present systems. The temperature is controlled at 30°C by cooling the recycle loop. The carbon dioxide coming from the fermentor is cooled and stripped of ethanol. In this process it is assumed that the carbon dioxide has no value, and so is vented.

f) Cell Separation.

The beer leaving the fermentor is sent to the cross-flow microfilters, white the cell-free filtrate stream is sent to the distillation column. A small bleed fraction is removed from the yeast concentrate stream with the rest being

returned to the fermentor.

g) Dried Yeast Preparation.

The excess yeast produced by the fermentation is then dried and sold as an animal feed. The bleed stream is first passed through a decanter centrifuge so that most of the ethanol in this stream can be recovered. The liquid from the centrifuge then goes to the distillation stage. The yeast solids, at around 30 % dry weight, are then dried to 90 % solids.

h) Distillation.

Azeotropic distillation is used for the production of anhydrous ethanol. Three columns are used; a stripper-rectifier, a dehydration tower and an ether stripper. The beer coming from the cell separation stage contains around 7.4 % (wt./vol) ethanol and first goes to a holding tank. It then goes through a series of preheating steps in which the beer is heated to saturation at 4.4 bar in a series of heat exchangers. The beer then enters the middle of the pressurized stripper-rectifier column. The bottoms from this column contain around 0.02 % (wt/vol) ethanol and leave around 151° C. It is then flashed to 100°C, with the vapour being used in the preheat and sent to the evaporator storage tank. A 12 bar forced recirculation reboiler is used at the bottom of the column. Fusel oils are removed as a liquid product about half way up the rectifier and then washed with water to remove any ethanol. The vapours from the top of the column are used to heat the feed by condensation and then (except for a small purge to remove light ends) are totally recycled back to the column. The liquid product is drawn off five trays below the top of the column and is approximately 94 % ethanol at a temperature of around 125°C. The liquid then enters the dehydration tower,

which is operated at atmospheric pressure. The ethanol stream comes off the bottom at 99.2 % (wt./wt.) ethanol, is cooled and sent to storage. The top stream is condensed, providing heat for the feed preheat, cooled and mixed with the top stream from the ether stripper in a decanter. The upper (ether) layer of this is sent to the top tray of the dehydration tower, while the lower layer goes to the ether stripper column. The bottom stream from the ether stripper is almost all water, which goes to the waste water plant while the top stream is cooled and returned to the decanter.

i) Evaporation.

From the evaporation storage tank, a fraction of the liquid is sent as backset, while the rest is evaporated to give a 60 %wt 'solubles' solution. To save energy, a vapour recompression evaporator was chosen. This uses 41 bar steam from the boiler to drive the compressor turbine, with the vapour from the top of the evaporator being compressed to 1.45 bar. The steam from the turbine leaves at 12 bar and is then used in the cooking and distillation stages.

j) Backset.

The fraction of backset commonly used is between 10 - 30 % of the mash, although values as high as 70 % (Ronkainen et al., 1978) have been suggested and 60 % has been used at the Pekin continuous fermentation plant (Allen, 1984). Here, a value of 40 % is used, although a higher value may be possible in actual operating conditions.

k) Dried Grain and Dried Solubles Drying.

The nonfermentable solids in a fermentation process are usually sold as a

dried mixture of the suspended and soluble solids. In this process, as the dried grains are separated before fermentation and distillation, a minimum of damage to their biological properties occurs (Loser, 1980). A rotary, brick-lined dryer is used for drying, with the grains being tumbled with the hot flue gases from the boiler. This gas enters at approximately 320°C and the gas and grains leave at around 90°C. To keep the dryer operating efficiently, the feed needs to be at least 70 % solids, so approximately 65 % of the grains coming from the dryer outlet steam are recycled back into the dryer. The final product at 90 % solids is then cooled in an auger-type heat exchanger and sent to storage.

l) Product Storage

Storage tanks in two separate buildings for 28 days ethanol production and 7 days dried grains and solubles are used. One per cent gasoline is added to the ethanol at this stage as a denaturant.

m) Utilities

A plant this size has large water requirements for cooling and much of this is provided by reusing water cooled in a water tower. However, fresh cold water, especially for final cooling is also required. This cooling water is itself then used as makeup water for that lost in the cooling tower and for the water in the process. The water coming from the evaporator and from the ether stripping tower is high in BOD and is treated in a standard aerobic waste water treatment plant.

Steam at 41 bar is provided by a gas-fired boiler operated at 77 % efficiency so as to provide flue gases for the dryer. An external supply of electricity is assumed available. A fire protection system with an underground distribution system and hydrants is also required.

Plant Costing

The costing of the process was based on the report by R. Katzen and Assoc. (1978), except for the saccharification column (Nyiri and Charles, 1977)), the fermentor (Maiorella et al., 1983)), the membrane filters (A/G Technol., 1991) and the distillation columns, driers and boilers (Ulrich, 1984). These prices were then converted to 1990 values using the Chemical Engineering Price Index (Anonymous, 1990a) and to the required size using size exponents from Garrett (1989) and Kalk and Langlykke (1986). The prices of raw materials, energy sources and products used in the design were taken for values from Saskatchewan in early 1991 and along with some of the calculated process yields are given in Table B1. Although its value may be higher than Distiller's Dried Grain (because the dried grains by-product is separated before processing) a similar value is assumed here.

Table B1 Important raw material, utility and product costs and process yields used in the costing of the 10^8 L/yr. ethanol production facility.

RAW MATERIAL	COST $CAN	UTILITY	COST $CAN	PRODUCT	COST $CAN
WHEAT † /T (62.6 % STARCH)	90	NATURAL GAS † /1000 M^3	100	ETHANOL ‡ /1000L	460
GELATINIZATION ENZYMES /T	5000	ELECTRICITY † /Gwh	250	DRIED GRAINS † /T	180
AMMONIA /T	250	WATER † /1000 M^3	4	DRIED YEAST • /T	700

STAGE	COOKING	SUSPENDED SOLIDS SEPN.	SACCHARIFICATION	FERMENTATION	OVERALL
% YIELD BY WEIGHT	98 STARCH TO DEXTRINS	97 DEXTRINS RECOVERED	97 DEXTRINS TO UTILIZABLE SUGARS	46 SUGARS TO ETHANOL 5 SUGARS TO YEAST	28 WHEAT TO DRIED GRAINS

† Costs taken From values for Saskatoon
‡ Anonymous (1991a)
• Anonymous (1991b)

The estimated capital costs, utilities and personnel for the new design, assuming the flows and concentrations shown in Figure B1, are shown in Tables B2, B3 and B4. Table B5 then gives an estimated breakdown of the expenses and revenues from the plant, both per hour and per 1000 L of ethanol produced.

Table B2 Estimated capital costs (in $\$10^6$ CAN) for the proposed 100 ML/year ethanol plant.

STAGE	EQUIPMENT	OTHER DIRECT COSTS	INDIRECT COSTS	TOTAL
FEED STORAGE	1.0	0.7	0.5	2.2
COOKING	0.8	1.3	0.7	2.8
CENTRIFUGES	1.2	0.5	0.6	2.3
SACCHARIFICATION	0.8	0.2	0.3	1.3
FERMENTATION	0.6	0.3	0.3	1.2
CELL SEPARATION	1.0		0.1	1.1
DRIED YEAST PROCESSING	0.3	0.1	0.1	0.5
DISTILLATION	1.8	2.0	1.5	5.3
EVAPORATION	3.9	1.6	0.9	6.4
DRIED GRAIN PROCESSING	1.3	1.8	1.5	4.6
PRODUCT STORAGE	0.7	0.9	0.6	2.2
BOILER	2.3	0.8	0.8	3.9
COOLING TOWER	0.4	0.2	0.1	0.7
WASTE WATER TREATMENT & SEWAGE				2.8
PIPING, GRIDS & FIRE				3.8
GENERAL SERVICES				2.0
TOTAL FIXED CAPITAL	16.1	10.4	8.0	43.1
CONTINGENCY				4.3
WORKING CAPITAL				3.0
TOTAL CAPITAL COST				50.4

Table B3 Estimated utilities required to operate the proposed 10^8 L/year Ethanol Plant. a) Steam produced (+) and used (-); b) Electricity and Cooling Water used.

a)

STAGE	STEAM UTILIZATION OF EACH STAGE (TONNES/H)			
	41 BAR STEAM	12 BAR STEAM	2 BAR STEAM	CONDENSATE
BOILER	+ 41.6			- 27.7
COOKING		- 13.9	+ 4.0	
DISTILLATION		- 27.7	- 4.0	+ 27.7
EVAPORATION	- 41.6	+ 41.6		

b)

	ELECTRICITY (KW)	COOLING WATER (15° C) (TONNES/H)
STORAGE & MILLING	270	
COOKING	110	90
CENTRIFUGATION	1030	
SACCHARIFICATION	20	230
FERMENTATION	120	
CELL SEPARATION	500	
DISTILLATION	70	
EVAPORATION & DRYING	320	20
PRODUCT STORAGE	40	
UTILITIES	700	
TOTAL	3180	340

Table B4 Estimated personnel costs ($/year) for the proposed 100 ML/year ethanol plant; based on 4 shifts operating 24 h/day, 7 days/week.

PERSONNEL TYPE	AVERAGE COST INCLUDING BENEFITS	TOTAL
PROFESSIONAL - 3 MANAGERS, 2 ENGINEERS, 1 CHEMIST	45,000	270,000
SKILLED - 4 TECHNICIANS, 12 MAINTENANCE STAFF, 16 OPERATORS, 4 NURSES	40,000	1,440,000
UNSKILLED - 2 SECRETARIES, 4 GUARDS, 12 LABOURERS	30,000	540,000
TOTAL		2,250,000

Table B5 Breakdown of estimated expenditures and incomes for the proposed 100 ML/year ethanol plant

EXPENDITURE	$/h	$/1000 L ETHANOL
WHEAT	3623	285
OTHER RAW MATERIALS	300	24
NATURAL GAS	370	29
ELECTRICITY	55	4
WATER	30	3
PERSONNEL	285	22
MAINTENANCE	190	15
INSURANCE & LICENCES	45	4
PAYMENT ON CAPITAL (10 YEARS INCLUDING 15 % INTEREST)	1200	93
TOTAL EXPENDITURE	6098	479

INCOME	$/h	$/1000L ETHANOL
ETHANOL	5842	460
YEAST	840	66
DDGS	1890	149
TOTAL INCOME	8572	675

APPENDIX B REFERENCES

Allen, A. S., in *8th Energy Biomass Wastes*, Klass, D. L., Ed., (Inst. Gas Technol.,; Chicago), 1223 - 35, 1984.

Anonymous, *Chemical Engineering*, **98(4)**, 202, 1990.

Anonymous, *Chemical Marketing Reporter*, Jan. 14, 1991a.

Anonymous, *Feedstocks*, 63, Jan. 7, 1991b.

Garrett, D. E., *Chemical Engineering Economics*, (Van Nostrand Reinhold, New York). 1989

Kalk, J. P., and Langlykke, A. F., in *Manual of Industrial Microbiology and Biotechnology*, Demain, A. L., and Solomon, N. A., Eds., (Am. Soc. Micro., Washington) 363, 1986.

Katzen, R., and Assoc., *Grain Motor Fuel Alcohol Technical and Economic Assessment Study*. (U.S. Dept. of Energy, Washington) 1978.

Loser, R. F., *Dev. Ind. Micro.*, **22**, 125, 1980.

Maiorella, B. L., Blanch, H. W., and Wilke, C. R., *Biotechnol. Bioeng.*, **26**, 1003, 1983.

Nyiri, L. K., and Charles, M. *Ann. Rep. Ferm. Proc.*, 1, 365, 1977.

Ronkainen, P., Leppanen, O., and Harju, K., *J. Inst. Brew.* **84**, 115, 1978.

Ulrich, G. D., *A Guide to Chemical Engineering Process Design and Economics*, (John Wiley & Sons, New York) 1984.